PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 24 October 2000 (24.10.00)	
International application No. PCT/US00/01338	**Applicant's or agent's file reference** 3260.93-304
International filing date (day/month/year) 21 January 2000 (21.01.00)	**Priority date** (day/month/year) 21 January 1999 (21.01.99)
Applicant CERRETTI, Douglas, Pat	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

18 August 2000 (18.08.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below
3260.93-304		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/01338	21/01/2000	21/01/1999
Applicant		
IMMUNEX CORPORATION et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[ ] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2 [ ] **Certain claims were found unsearchable** (See Box I)

3 [X] **Unity of invention is lacking** (see Box II)

4 With regard to the **title,**

[X] the text is approved as submitted by the applicant

[ ] the text has been established by this Authority to read as follows

5 With regard to the **abstract,**

[X] the text is approved as submitted by the applicant

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6 With regard to the drawings

[X] as suggested by the applicant

[ ] because the applicant failed to suggest a figure

[ ] because this figure better characterizes the invention

[ ] None of the figures

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/01338

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Claims 1-14.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-14

SVPH1, its encoding nucleotides and antibodies against SVPH1

2. Claims: 15-28

SVPH4, its encoding nucleotides and antibodies against SVPH4

3. Claims: 29-42

SVPH3, its encoding nucleotides and antibodies against SVPH3

INTERNATIONAL SEARCH REPORT

National Application No
PCT/US 00/01338

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/57 C12N9/64 C07K16/40 C12N1/21 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL/GENBANK DATABASES Accession no X85598 Sequence reference HS111AEST JONES M ET AL: "Chromosomal assignmentof 3XX sequences transcribed in adult human testis" XP002137513 the whole document ---	1-14
A	HOOFT VAN HUIJSDUIJNEN R: "ADAM 20 and 21;two novel human testis -specific membrane metalloproteases with similarity to fertilin-alpha" GENE,NL,ELSEVIER, AMSTERDAM, vol. 206, no. 2, 12 January 1998 (1998-01-12), pages 273-282, XP002088618 ISSN: 0378-1119 --- -/--	

[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex

° Special categories of cited documents

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

01 09 00

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040

Authorized officer

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/01338

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
P,A	WO 99 36549 A (IMMUNEX CORP ;CERRETTI DOUGLAS PAT (US)) 22 July 1999 (1999-07-22) ---	
P,X	CERRETTI DOUGLAS PAT ET AL: "Isolation of two novel metalloproteinase-disintegrin (ADAM) cDNAs that show testis-specific gene expression." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS OCT. 5, 1999, vol. 263, no. 3, 5 October 1999 (1999-10-05), pages 810-815, XP002137511 ISSN: 0006-291X the whole document ---	1-14
P,X	XU RENER ET AL: "Molecular cloning and mapping of a novel ADAM gene (ADAM29) to human chromosome 4." GENOMICS DEC. 15, 1999, vol. 62, no. 3, 15 December 1999 (1999-12-15), pages 537-539, XP002137512 ISSN: 0888-7543 the whole document -----	1-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/01333

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9936549 A	22-07-1999	AU 2221999 A	02-08-1999

PATENT COOPERATION TF

EL591094879US

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.
Attn. Garrett, Arthur, S.
1300 I Street, N.W.
Washington, D.C. 20005-3315
UNITED STATES OF AMERICA

Date of mailing *(day/month/year)* 01/09/2000	
Applicant's or agent's file reference 3260.93-304	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 00/01338	International filing date *(day/month/year)* 21/01/2000
Applicant IMMUNEX CORPORATION et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46).

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

RECEIVED
SEP 18 2000
FINNEGAN, HENDERSON, FARABOW GARRETT AND DUNNER, LLP

For more detailed instructions, see the notes on the accompanying sheet

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis* 1 and 90*bis* 3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant

Name and mailing address of the International Searching Authority: European Patent Office, P.B. 5818 Patentlaan 2, NL-2280 HV Rijswijk	Authorized officer: Andria Overbeeke-Siepker

ARTICLE 19 Due 11/1/0

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2)

Where a demand for international preliminary examination has been/is filed, see below

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b))

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter

[illegible] application. However, if the language of the international application is English, the letter must be in the language of the international application. If French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged,

(ii) the claim is cancelled,

(iii) the claim is new,

(iv) the claim replaces one or more claims as filed,

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1))

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the [illegible]

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 3260.93-304	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/01338	International filing date *(day/month/year)* 21/01/2000	Priority date *(day/month/year)* 21/01/1999
International Patent Classification (IPC) or national classification and IPC C12N15/57		
Applicant IMMUNEX CORPORATION et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Name and mailing address of the international preliminary examining authority:

Authorized officer

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/01338

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-70 as originally filed

Claims, No.:

1-44 as received on 14/02/2001 with letter of 14/02/2001

Drawings, sheets:

1/2,2/2 as originally filed

Sequence listing part of the description, pages:

1-26, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
see separate sheet

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 15-42,44.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 15-42,44.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability, citations and explanations supporting such statement

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/01338

1. Statement

Novelty (N)	Yes:	Claims	1(a)(b)(e),2-14
	No:	Claims	43
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1(a)(b)(e),2-14,43
Industrial applicability (IA)	Yes:	Claims	1(a)(b)(e),2-14, 43
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

Basis of the report

1. The amendments filed with the letter dated 14.02.01 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are in claims 1, 13, 15 and 27.

   Page 23, lines 8-17 discloses that each of the SVPH-1 polypeptides encodes a signal sequence, a pro-domain, a catalytic domain, a disintegrin domain, a cysteine rich region **and** a transmembrane domain; but no basis is provided for nucleic acid molecules encoding polypeptide **fragments** comprising iess than aii these domains, for instance only one domain. Therefore, said passage of the description does not provide a basis for amended claims 1(c) and 13.

   Page 45, lines 10-13 refers to nucleotide fragments comprising at least about 17 contiguous nucleotides of a DNA sequence according to the application, but does not generally disclose nucleic acid molecules comprising at least about 17 contiguous nucleotides which hybridize to nucleic acid molecules according to the application. It is important to note that it is not sufficient that the features of a claimed molecule as such are originally disclosed, but their combination has to be disclosed as well. Moreover, claim 1 (d) refers to claim 1 (c) which is in any case not based on the application as filed.

   Consequently, the examination was limited to claim 1 (a),(b),(e), to claim 13 as far as it relates to a polypeptide comprising an amino acid sequence selected from the group of SEQ ID NO: 12, 13 and 14, and to claim 43. Claims 3-12 and 14 have only been examined to the extend to which they refer back to these parts of claims 1 and 13.

   Accordingly, claims 15 and 27 also go beyond the disclosure of the application as originally filed; however these claims have not been subject to examination

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2. The following documents are cited:

D1: EMBL/GENBANK DATABASES Accession no X85598 Sequence reference HS111AEST JONES M ET AL: 'Chromosomal assignment of 3XX sequences transcribed in adult human testis'
D2: HOOFT VAN HUIJSDUIJNEN R: GENE, vol. 206, no. 2, 12 January 1998 (1998-01-12), pages 273-282
D3: WO 99 36549 A
D4: CERRETTI DOUGLAS PAT ET AL: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS OCT. 5, vol. 263, no. 3, 5 October 1999 (1999-10-05), pages 810-815
D5: XU RENER ET AL: GENOMICS DEC. 15, 1999, vol. 62, no. 3, 15 December 1999 (1999-12-15), pages 537-539

3. The current assessment is based on the assumption that all claims enjoy priority rights from the filing date of the priority document. If it later turns out that this is not correct, the documents D3 to D5 cited in the international search report would become relevant.

4. For the purpose of the present report, the (unclear) claim 43 has been examined as if it was directed to the subject-matter of claim 1 (d) and (f) as originally filed (which was apparently intended by the Applicant). Claim 43 is broader in scope that amended claim 1 to which it refers, and it would therefore not appear to be meaningful to examine it as dependent claim.

Based on this interpretation, the subject-matter of claim 43 is not new, because it does not clearly define the claimed nucleic acid molecule. Claim 43(a) encompasses **any** nucleic acid encoding any amino acid sequence since any

43(b) do also not define clear features which could distinguish the claimed

molecules from the prior art ones. Therefore, claim 43 is considered to lack novelty, in particular over the document D2 which discloses polypeptides with more than 50% amino acid identity to those of the application, but also over any other known nucleic acid molecule.

5. Claim 1(a)(b)(e), claims 2-12 and 14 referring thereto, and claim 13 as far as it relates to a polypeptide comprising an amino acid sequence selected from the group of SEQ ID NO: 12, 13 and 14 appear to be novel over the available prior art, since the claims do no longer refer to SEQ ID NO:1 known from D1.

However, these claims are not considered to involve an inventive step for the following reasons.

The present application does provide no evidence of the activity or biological role of the claimed molecules. Consequently, the invention of the present application is considered merely to be the provision of a transcribed sequence with no known technical useful property. In this case, **any** prior art compound (e.g. those of D2) is equally suitable as the starting point for making structural modifications and may be considered as the "closest prior art".
Starting from this point, the only technical problem which may be derived is the provision of a further compound as such, regardless of its useful properties.
Without the concomitant need to provide any particular technical effect, the skilled person would have had the choice of an infinite number of equally possible solutions. An arbitrary selection from this host of possible solutions cannot involve an inventive step because, in order to be inventive, the selection must not be arbitrary but must be justified by the technical purpose, i.e. by a hitherto unknown or unexpected technical effect which is caused by those structural features distinguishing the claimed compound from the numerous other ones.
The Applicant's argument that the technical problem of the application should be seen in the disclosure of a metalloproteinase-disintegrin polypeptide could not be followed, since no such proteinase activity has been demonstrated for the polypeptides according to the application. Only a problem which has been actually

Re Item VI

Certain documents cited

6. Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 99/36549	22.07.99	12.01.99	14.01.98

Re Item VIII

Certain observations on the international application

7. The application does not meet the requirements of Article 6 PCT because the claims do not clearly define the subject-matter claimed. Claim 43(a) encompassed nearly any known nucleic acid molecule (see also point 4. above). Furthermore, the terms "SVPH", "SVPH1", "allelic variant" and "species homolog" used in claim 1, and the term "SVPH1 polypeptide" used in claim 43(b) are vague and unclear and leave the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT). The term "about" renders claim 1(d) unclear.

PATENT COOPERATION TREATY

BF/MT EI 5910948 79US

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

MAY 03 2001

PCT

FINNEGAN, HENDERSON,
FARABOW, GARRETT & DUNNER, L.L.P.

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT
(PCT Rule 71.1)

To:

Garrett, Arthur, S.
FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.
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Date of mailing *(day/month/year)*	25.04.2001
Applicant's or agent's file reference: 3260.93-304	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/01338	21/01/2000	21/01/1999

Applicant
IMMUNEX CORPORATION et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes, at least one must be marked)*:

Regional Patent

☒ **AP ARIPO Patent**: **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SL** Sierra Leone, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ **EA Eurasian Patent**: **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of

☒ **EP European Patent**: **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ **OA OAPI Patent**: **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Cote d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ **AE** United Arab Emirates
☒ **AL** Albania .....
☒ **AM** Armenia .....
☒ **AT** Austria .....
☒ **AU** Australia .....
☒ **AZ** Azerbaijan
☒ **BA** Bosnia and Herzegovina .....
☒ **BB** Barbados
☒ **BG** Bulgaria .....
☒ **BR** Brazil .....
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☒ **CA** Canada
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☒ **HU** Hungary
☒ **ID** Indonesia
☒ **IL** Israel
☒ **IN** India
☒ **IS** Iceland
☒ **JP** Japan .....
☒ **KE** Kenya .....
☒ **KG** Kyrgyzstan .....
☒ **KP** Democratic People's Republic of Korea .....
☒ **KR** .....
☒ **LR** Liberia
☒ **LS** Lesotho .....
☒ **LT** Lithuania
☒ **LU** Luxembourg
☒ **LV** Latvia
☒ **MD** Republic of Moldova .....
☒ **MG** Madagascar .....
☒ **MK** The former Yugoslav Republic of Macedonia .....
.....
☒ **MN** Mongolia
☒ **MW** Malawi .....
☒ **MX** Mexico .....
☒ **NO** Norway
☒ **NZ** New Zealand .....
☒ **PL** Poland .....
☒ **PT** Portugal .....
☒ **RO** Romania
☒ **RU** Russian Federation .....
☒ **SD** Sudan
☒ **SE** Sweden
☒ **SG** Singapore
☒ **SI** Slovenia .....
☒ **SK** Slovakia .....
☒ **SL** Sierra Leone
☒ **TJ** Tajikistan .....
☒ **TM** Turkmenistan .....
☒ **TR** Turkey .....
☒ **TT** Trinidad and Tobago .....
☒ **UA** Ukraine .....
☒ **UG** Uganda .....
☒ **US** United States of America .....
.....
☒ **UZ** Uzbekistan .....
☒ **VN** Viet Nam .....
☒ **YU** Yugoslavia .....
☒ **ZA** South Africa .....
☒ **ZW** Zimbabwe

☒ [illegible] ☒ [illegible]

Precautionary Designation Statement: [illegible] designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and [illegible]

Supplemental Box *If the Supplemental Box is not used, this sheet need not be included in the request*

*1. If, in any of the Boxes, **the space is insufficient** to furnish all the information in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:*

*(i) **if more than two persons are involved as applicants and/or inventors** and no "continuation sheet" is available, in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;*

*(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication **"the States indicated in the Supplemental Box"** is checked; in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*

*(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, **the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America:** in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*

*(iv) if, in addition to the agent(s) indicated in Box IV, there are **further agents**: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*

*(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication **"patent of addition,"** or **"certificate of addition,"** or if, in Box No. V, the name of the United States of America is accompanied by an indication **"continuation"** or **"continuation-in-part"**: in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*

*(vi) if, in Box No. VI, there are **more than three earlier applications whose priority is claimed:** in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;*

*(vii) if, in Box No. VI, the **earlier application is an ARIPO application:** in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.*

*2. If, with regard to the **precautionary designation statement** contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.*

*3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning **non-prejudicial disclosures or exceptions to lack of novelty**: in such case, write "Statement concerning non prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.*

CONTINUATION OF BOX IV:

VOIGHT, Jerry D.
PETERSON, Stephen L.
JENNINGS, Tipton D.
SANTORELLI, Albert J.
GRIFFEN, Susan H.
ZOTTER, Bruce C.
GRAHAM, Barry W.
CONVERSE, Robert E.
CHAPMAN, Ernest F.
FORDIS, Jean Burke

all of FINNEGAN, HENDERSON, FARABOW, GARRET & DUNNE, L.L.P.
1300 I Street, N.W., Washington, DC 20005-3315
United States of America

What is claimed is:

1. An isolated SVPH nucleic acid molecule selected from the group consisting of:

(a) the DNA sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9;

(b) an isolated nucleic acid molecule encoding an amino acid sequence comprising the sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14;

(c) an isolated nucleic acid molecule that hybridizes to either strand of a denatured, double-stranded DNA comprising the nucleic acid sequence of (a) or (b) under conditions of moderate stringency in 50% formamide and 6XSSC, at 42°C with washing conditions of 60°C, 0.5XSSC, 0.1% SDS;

(d) an isolated nucleic acid molecule derived by *in vitro* mutagenesis from SEQ ID NO:1, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9;

(e) an isolated nucleic acid molecule degenerate from SEQ ID NO:1, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9 as a result of the genetic code; and

(f) an isolated nucleic acid molecule selected from the group consisting of human SVPH 1 DNA; an allelic variant of human SVPH 1 DNA; and a species homolog of SVPH 1 DNA.

2. The nucleic acid molecule of claim 1 selected from the group consisting of SEQ ID NO:1, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9.

3. A recombinant vector that directs the expression of the nucleic acid molecule of claim 1.

4. An isolated polypeptide encoded by the nucleic acid molecule of claim 1.

5. An isolated polypeptide according to claim 4 having a molecular weight selected from the group consisting of approximately 4,199; 86,983; 89,459.

6. Isolated antibodies that bind to a polypeptide of claim 4.

7. Isolated antibodies according to claim 7, wherein the antibodies are

9. A host cell transfected or transduced with the vector of claim 3.

10. A method for the production of SVPH-1 polypeptide comprising culturing a host cell of claim 9 under conditions promoting expression, and recovering the polypeptide from the culture medium.

11. The method of claim 10, wherein the host cell is selected from the group consisting of bacterial cells, yeast cells, plant cells, and animal cells.

12. The method of claim 10, wherein the host cell is a mammalian cell.

13. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14.

14. An oligomer comprising a polypeptide of claim 4.

15. An isolated SVPH nucleic acid molecule selected from the group consisting of:

(a) the DNA sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:10, and SEQ ID NO:11;

(b) an isolated nucleic acid molecule encoding an amino acid sequence comprising the sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:15, and SEQ ID NO:16;

(c) an isolated nucleic acid molecule that hybridizes to either strand of a denatured, double-stranded DNA comprising the nucleic acid sequence of (a) or (b) under conditions of moderate stringency in 50% formamide and 6XSSC, at 42°C with washing conditions of 60°C, 0.5XSSC, 0.1% SDS;

(d) an isolated nucleic acid molecule derived by *in vitro* mutagenesis from SEQ ID NO:3, SEQ ID NO:10, and SEQ ID NO:11;

(e) an isolated nucleic acid molecule degenerate from SEQ ID NO:3, SEQ ID NO:10, and SEQ ID NO:11 as a result of the genetic code; and

(f) an isolated nucleic acid molecule selected from the group consisting of human SVPH-4 DNA, an allelic variant of human SVPH-4 DNA [illegible]

16. The nucleic acid molecule of claim 15 selected from the group consisting of SEQ ID NO:3, SEQ ID NO:10, and SEQ ID NO:11.

17. A recombinant vector that directs the expression of the nucleic acid molecule of claim 15.

18. An isolated polypeptide encoded by the nucleic acid molecule of claim 15.

19. An isolated polypeptide according to claim 18 having a molecular weight selected from the group consisting of approximately 55,209; 88,923; and 87,990 Daltons as determined by SDS-PAGE.

20. An isolated polypeptide according to claim 18 in non-glycosylated form.

21. Isolated antibodies that bind to a polypeptide of claim 18.

22. Isolated antibodies according to claim 21, wherein the antibodies are monoclonal antibodies.

23. A host cell transfected or transduced with the vector of claim 17.

24. A method for the production of SVPH 4 polypeptide comprising culturing a host cell of claim 23 under conditions promoting expression, and recovering the polypeptide from the culture medium.

25. The method of claim 24, wherein the host cell is selected from the group consisting of bacterial cells, yeast cells, plant cells, and animal cells.

26. The method of claim 24, wherein the host cell is a mammalian cell.

27. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:15, and SEQ ID NO:16.

28. An oligomer comprising a polypeptide of claim 18.

29. An isolated SVPH nucleic acid molecule selected from the group consisting of:

(a) the DNA sequence of SEQ ID NO:2;

(b) [illegible] nucleic acid that hybridizes to either strand of a denatured, double stranded DNA comprising the nucleic acid sequence of (a) or (b)

under conditions of moderate stringency in 50% formamide and 6XSSC, at 42°C with washing conditions of 60°C, 0.5XSSC, 0.1% SDS;

(d) an isolated nucleic acid molecule derived by *in vitro* mutagenesis from SEQ ID NO:2;

(e) an isolated nucleic acid molecule degenerate from SEQ ID NO:2 as a result of the genetic code; and

(f) an isolated nucleic acid molecule selected from the group consisting of human SVPH 3 DNA; an allelic variant of human SVPH 3 DNA; and a species homolog of SVPH 3 DNA.

30. The nucleic acid molecule of claim 29, wherein the DNA sequence comprises SEQ ID NO:2.

31. A recombinant vector that directs the expression of the nucleic acid molecule of claim 29.

32. An isolated polypeptide encoded by the nucleic acid molecule of claim 29.

33. An isolated polypeptide according to claim 32 having a molecular weight of approximately 13,938 Daltons as determined by SDS-PAGE.

34. An isolated polypeptide according to claim 32 in non-glycosylated form.

35. Isolated antibodies that bind to a polypeptide of claim 32.

36. Isolated antibodies according to claim 35, wherein the antibodies are monoclonal antibodies.

37. A host cell transfected or transduced with the vector of claim 31.

38. A method for the production of SVPH 3 polypeptide comprising culturing a host cell of claim 37 under conditions promoting expression, and recovering the polypeptide from the culture medium.

39. The method of claim 38, wherein the host cell is selected from the group consisting of bacterial cells, yeast cells, plant cells, and [illegible]

[illegible]

[illegible]

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 July 2000 (27.07.2000)

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(10) International Publication Number
WO 00/43525 A3

(51) International Patent Classification[7]: C12N 15/57, 9/64, C07K 16/40, C12N 1/21, 5/10

(21) International Application Number: PCT/US00/01338

(22) International Filing Date: 21 January 2000 (21.01.2000)

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(30) Priority Data:

60/116,670	21 January 1999 (21.01.1999)	US
60/138,682	14 June 1999 (14.06.1999)	US
60/155,798	27 September 1999 (27.09.1999)	US

(71) Applicant *(for all designated States except US)*: IMMUNEX CORPORATION [US/US]; 51 University Street, Seattle, WA 98101 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: CERRETTI, Douglas, Pat [US/US]; 1607 North 197th Place, Seattle, WA 98133 (US).

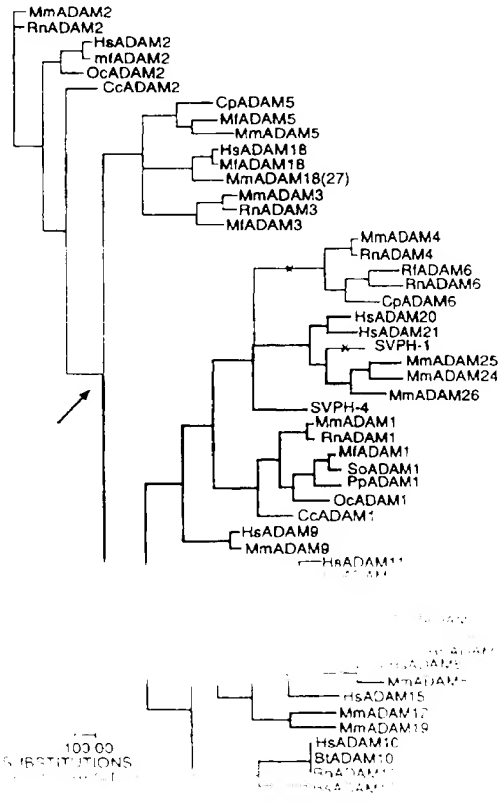
(74) Agents: GARRETT, Arthur, S. et al.; Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P., 1300 I Street, N.W., Washington, DC 20005-3315 (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: METALLOPROTEINASE-DISINTEGRIN FAMILY MEMBERS: SVPH DNAS AND POLYPEPTIDES



(57) Abstract: The invention is directed to purified and isolated novel SVPH polypeptides, the nucleic acids encoding such polypeptides, processes for production of recombinant forms of such polypeptides, and the uses of the above.

WO 00/4 5 A3

Published:
— *With international search report.*

(88) Date of publication of the international search report:
30 November 2000

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/01338

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/57 C12N9/64 C07K16/40 C12N1/21 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL/GENBANK DATABASES Accession no X85598 Sequence reference HS111AEST JONES M ET AL: "Chromosomal assignmentcf 3XX sequences transcribed in adult human testis" XP002137513 the whole document ---	1-14
A	HOOFT VAN HUIJSDUIJNEN R: "ADAM 20 and 21;two novel human testis -specific membrane metalloproteases with similarity to fertilin-alpha" GENE,NL,ELSEVIER, AMSTERDAM, vol. 206, no. 2, 12 January 1998 (1998-01-12), pages 273-282, XP002088618 ISSN: 0378-1119 --- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 May 2000	01.09.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo n Fax (+31-70) 340-3016	VAN DER SCHAAF, E.A.

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	WO 99 36549 A (IMMUNEX CORP ;CERRETTI DOUGLAS PAT (US)) 22 July 1999 (1999-07-22) ---	
P,X	CERRETTI DOUGLAS PAT ET AL: "Isolation of two novel metalloproteinase-disintegrin (ADAM) cDNAs that show testis-specific gene expression." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS OCT. 5, 1999, vol. 263, no. 3, 5 October 1999 (1999-10-05), pages 810-815, XP002137511 ISSN: 0006-291X the whole document ---	1-14
P,X	XU RENER ET AL: "Molecular cloning and mapping of a novel ADAM gene (ADAM29) to human chromosome 4." GENOMICS DEC. 15, 1999, vol. 62, no. 3, 15 December 1999 (1999-12-15), pages 537-539, XP002137512 ISSN: 0888-7543 the whole document -----	1-14

INTERNATIONAL SEARCH REPORT

International application No
PCT/US 00/01338

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3 ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4 ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest

☐ [illegible] payment of additional search fees

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 00/01338

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-14

   SVPH1, its encoding nucleotides and antibodies against SVPH1

2. Claims: 15-28

   SVPH4, its encoding nucleotides and antibodies against SVPH4

3. Claims: 29-42

   SVPH3, its encoding nucleotides and antibodies against SVPH3

INTERNATIONAL SEARCH REPORT

[redacted]ation on patent family members

[redacted]national Application No
[redacted]T/US 00/01338

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9936549 A	22-07-1999	AU 2221999 A	02-08-1999

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/57, 9/64, C07K 16/40, C12N 1/21, 5/10

A2

(11) International Publication Number: **WO 00/43525**

(43) International Publication Date: 27 July 2000 (27.07.00)

(21) International Application Number: PCT/US00/01338

(22) International Filing Date: 21 January 2000 (21.01.00)

(30) Priority Data:

60/116,670	21 January 1999 (21.01.99)	US
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(71) Applicant *(for all designated States except US)*: IMMUNEX CORPORATION [US/US]; 51 University Street, Seattle, WA 98101 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: CERRETTI, Douglas, Pat [US/US]; 1607 North 197th Place, Seattle, WA 98133 (US).

(74) Agents: GARRETT, Arthur, S. et al.; Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P., 1300 I Street, N.W., Washington, DC 20005-3315 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

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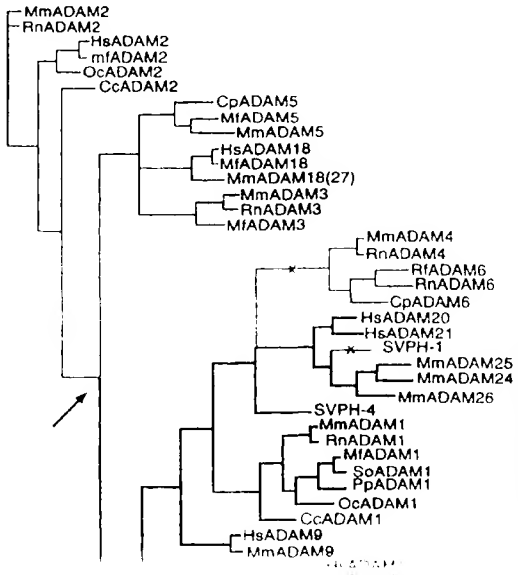
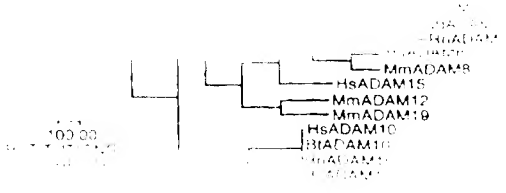
(54) Title: METALLOPROTEINASE-DISINTEGRIN FAMILY MEMBERS: SVPH DNAS AND POLYPEPTIDES

(57) Abstract

The invention is directed to purified and isolated novel SVPH polypeptides, the nucleic acids encoding such polypeptides, processes for production of recombinant forms of such polypeptides, and the uses of the above.





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METALLOPROTEINASE-DISINTEGRIN FAMILY MEMBERS: SVPH DNAS AND POLYPEPTIDES

CROSS REFERENCE TO RELATED APPLICATIONS

This application hereby claims the benefit of United States provisional applications S.N. 60/116,670; S.N. 60/138,682; and S.N. 60/155,798; filed January 21, 1999; June 14, 1999; and September 27, 1999, respectively. The entire disclosures of these applications are relied upon and incorporated by reference herein.

BACKGROUND OF THE INVENTION

Field of the Invention

The invention is directed to purified and isolated, novel SVPH polypeptides (SVPH-1, SVPH-1a, SVPH-1b, SVPH-1c; SVPH-3; and SVPH-4, SVPH-4a, and SVPH-4b) and fragments thereof, the nucleic acids encoding such polypeptides, processes for production of recombinant forms of such polypeptides, antibodies generated against these polypeptides, fragmented peptides derived from these polypeptides, and uses thereof.

Description of Related Art

Metalloproteinases are a group of proteinases characterized by the presence of a metal prosthetic group. Despite this basic similarity, the group, which includes proteinases from snake venom, numerous microbial proteinases, and vertebrate and bacterial collagenases, would seem to present proteinases of seemingly widely varying activities. For example, snake venom proteases are metalloproteinases that affect cell-matrix interactions. Snake venom also includes "disintegrins," a class of low molecular weight, Arg-Gly-Asp (RGD)-containing, cysteine-rich peptides which bind to integrins (a family of molecules involved in cell-to-cell adhesion, cell-to-matrix adhesion, and inflammatory responses) expressed on cells.

disintegrin-like, cysteine rich, and epidermal growth factor domains. See, Black et al., "ADAMs: focus on the protease domain," *Curr. Opin. Cell Biol.* 10:654-659 (1998);

401 (1996), all of which are herein incorporated by reference. The metalloproteinase-disintegrins or ADAMs have a unique domain structure composed of a signal sequence, pro-domain with a Cys switch, catalytic domain with a zinc-binding motif, disintegrin domain, cysteine-rich domain, a transmembrane domain, and a cytoplasmic domain (Black et al., "ADAMs: focus on the protease domain," *Curr. Opin. Cell Biol.* 10:654-659 (1998) ; Blobel, C. P., *Cell*, 90:589-592 (1997)). Thus, ADAMs are type 1 transmembrane proteins expressed on the cell surface. ADAMs have been isolated from mammalian species, *Caenorhabditis*, *Xenopus*, and *Drosophila*. Approximately half of the ADAMs do not contain the zinc-binding motif **HEXXHXXGXXHD** (SEQ ID NO:31), which is though to be required for enzymatic activity. However, all ADAMs contain the disintegrin domain, which is approximately 80 amino acids in length with 15 highly conserved Cys residues. In some members this region has been found to bind integrins (Almeida, E.A. et al., *Cell* 81:1095-1104 (1995); Zhang, X. P. et al., *J. Biol. Chem.* 273:7345-7350 (1998); Nath, D. et al., *J. Cell Sci.* 112:579-587 (1999)), although the role of this domain for the majority of the family members is unknown.

Over two dozen ADAMs have been identified but only a few have had their biological roles elucidated. Tumor necrosis factor-α converting enzyme (TACE/ADAM17) was isolated as the proteinase required for the shedding of TNF-α from the plasma membrane. *See*, Blobel, C.P, *Cell*, 90:589-592 (1997); Moss, M. et al., *Nature* 385:733-736 (1997); Black, R.A. et al., *Nature* 385:729-733 (1997). More recently TACE ADAM17 has been found to be required for the ectodomain shedding of other cell surface proteins including L-selectin, TGF-α, p80 TNFR, p60TNFR, L-selectin, type II IL-1R, and β-amyloid precursor protein (Peschon, J. J. et al., *Science* 282:1281-1284 (1998)). Fertilin-α/ADAM1 and fertilin-β/ADAM2 are required for sperm-egg fusion (Myles, D. G. et al., *Proc. Nat'l. Acad. Sci., USA* 91:4195-4198 (1994)) while meltrin-α ADAM12 has a role in muscle cell fusion (Yagami- [illegible]

[illegible] 280 (1997); Rooke, J. et al., *Science* 273:1227-1231 (1996)).

Some ADAMs are ubiquitously expressed such as ADAM9, ADAM10, ADAM15, and ADAM17 and may have pleiotropic effects, as has been found for ADAM15 and ADAM17. Many of the other ADAMs, however, show tissue-specific expression: ADAM12 and ADAM19 in muscle (Yagami-Hiromasa, T. et al., *Nature* 377:652-656 (1995)), ADAM22 in brain, and ADAM23 in brain and heart (Sagane, K. et al., *J. Biochem.* 334:93-98 (1998)). The largest group of ADAMs (Bjarnason, J. B. et al., *Methods Enzymol.* 248: 345-368 (1995); Jia, L. G. et al., *Toxicon* 34:1269-1276 (1996); Stocker, W. et al., *Protein Sci.* 4:823-840 (1995); Black, R. A. et al., *Curr. Opin. Cell Biol.* 10:654-659 (1998); Blobel, C. P. , *Cell* 90:589-592 (1997); Almeida, E. A. et al., *Cell* 81:1095-1104 (1995); Zhang, X. P. et al., *J. Biol. Chem.* 273:7345-7350 (1998); Wolfsberg, T. G. et al., *Dev. Biol.* 180:389-401 (1996); Zhu, G. Z. et al., *Gene* 234:227-237 (1999); Blobel, C. P. et al., *Nature* 356:248-252 (1992); Walter, M. A. et al., *Nat. Genet.* 7:22-28 (1994); Gribskov, M. et al., *Nucleic Acids Res.* 14:6745-6763 (1986); Bode, W. et al., *FEBS Lett.* 331:134-140 (1993); and Cerretti, D. P. et al., *Cytokine* 11:541-551 (1999)) is predominately expressed in testis and is thought to be involved in spermatogenesis and fertilization (Wolfsberg, T. G. et al., *Dev. Biol.* 180:389-401 (1996); Hooft van Huijsduijnen, R., *Gene* 206:273-282 (1998); Zhu, G. Z. et al., *Gene* 234:227-237 (1999)). Indeed, the first mammalian ADAMs discovered, ADAM1 and ADAM2, were found to be required for sperm-egg fusion (Zhu, G. Z. et al., *Gene* 234:227-237 (1999)).

The ADAMs family of metalloproteinase-disintegrins also share homology with the snake venom protease family (SVPH). In some snake venom protease members, the disintegrin domain prevents platelet aggregation and thus acts as an anti-coagulant.

Given the significant function of metalloproteinases in membrane and cell-cell fusion, cellular adhesion, shedding of membrane proteins, and anti-coagulation, there is a need in the art for additional metalloproteinases of the ADAMs family and or the SVPH family [illegible]

[illegible] unknown protein is the culmination of an arduous process of experimentation. In

order to identify an unknown protein, the investigator can rely upon a comparison of the unknown protein to known peptides using a variety of techniques known to those skilled in the art. For instance, proteins are routinely analyzed using techniques such as electrophoresis, sedimentation, chromatography, sequencing and mass spectrometry.

In particular, comparison of an unknown protein to polypeptides of known molecular weight allows a determination of the apparent molecular weight of the unknown protein (Brock, T. D. et al., *Biology of Microorganisms* 76-77 (1991)). Protein molecular weight standards are commercially available to assist in the estimation of molecular weights of unknown protein (New England Biolabs Inc. Catalog:130-131 (1995); J. L. Hartley, U.S. Patent No. 5,449,758). However, the molecular weight standards may not correspond closely enough in size to the unknown protein to allow an accurate estimation of apparent molecular weight. The difficulty in estimation of molecular weight is compounded in the case of proteins that are subjected to fragmentation by chemical or enzymatic means, modified by post-translational modification or processing, and/or associated with other proteins in non-covalent complexes.

In addition, the unique nature of the composition of a protein with regard to its specific amino acid constituents results in unique positioning of cleavage sites within the protein. Specific fragmentation of a protein by chemical or enzymatic cleavage results in a unique "peptide fingerprint" (Cleveland, D. W. et al., *J. Biol. Chem.* 252:1102-1106 (1977); Brown, M. et al., *J. Gen. Virol.* 50:309-316 (1980)). Consequently, cleavage at specific sites results in reproducible fragmentation of a given protein into peptides of precise molecular weights. Furthermore, these peptides possess unique charge characteristics that determine the isoelectric pH of the peptide. These unique characteristics can be exploited using a variety of electrophoretic and other techniques (Brock, T. D. et al., *Biology of Microorganisms* 76-77 (Prentice Hall, 6th ed. 1991)).

(1987); Eckerskorn, C. et al., *Electrophoresis 1988*, 9:830-838 (1988)), particularly

the production of fragments from proteins with a "blocked" N-terminus. In addition, fragmented proteins can be used for immunization, for affinity selection (R. A. Brown, U.S. Patent No. 5,151,412), for determination of modification sites (*e.g.* phosphorylation), for generation of active biological compounds (Brock, T. D. et al., *Biology of Microorganisms* 300-301 (Prentice Hall, 6th ed. 1991)), and for differentiation of homologous proteins (Brown, M. et al., *J. Gen. Virol.* 50:309-316 (1980)).

In addition, when a peptide fingerprint of an unknown protein is obtained, it can be compared to a database of known proteins to assist in the identification of the unknown protein using mass spectrometry (Henzel, W. J. et al., *Proc. Natl. Acad. Sci. USA* 90:5011-5015 (1993); Fenyo, D. et al., *Electrophoresis* 19:998-1005 (1998)). A variety of computer software programs to facilitate these comparisons are accessible via the Internet, such as Protein Prospector (Internet site: prospector.uscf.edu), MultiIdent (Internet site: www.expasy.ch/sprot/multiident.html), PeptideSearch (Internet site: www.mann.embl-heiedelberg.de...deSearch/FR_PeptideSearch Form.html), and ProFound (Internet site: www.chait-sgi.rockefeller.edu/cgi-bin/prot-id-frag.html). These programs allow the user to specify the cleavage agent and the molecular weights of the fragmented peptides within a designated tolerance. The programs compare these molecular weights to protein molecular weight information stored in databases to assist in determining the identity of the unknown protein. Accurate information concerning the number of fragmented peptides and the precise molecular weight of those peptides is required for accurate identification. Therefore, increasing the accuracy in determining the number of fragmented peptides and their molecular weight should result in enhanced likelihood of success in the identification of unknown proteins.

In addition, peptide digests of unknown proteins can be sequenced using tandem mass spectrometry (MS/MS) and the resulting sequence searched against databases (Eng, J. K. et al., *J. Am. Soc. Mass Spec.* 5:976-989 (1994)). [illegible] the Internet, such as Lutefisk 97 (Internet site: www.lsbc.com:70 Lutefisk97.html),

and the Protein Prospector, Peptide Search and ProFound programs described above. Therefore, adding the sequence of a gene and its predicted protein sequence and peptide fragments to a sequence database can aid in the identification of unknown proteins using tandem mass spectrometry.

Thus, there also exists a need in the art for polypeptides suitable for use in peptide fragmentation studies, for use in molecular weight measurements, and for use in protein sequencing using tandem mass spectrometry.

SUMMARY OF THE INVENTION

The invention aids in fulfilling these various needs in the art by providing isolated, novel SVPH nucleic acids and polypeptides encoded by these nucleic acids. Particular embodiments of the invention are directed to an isolated SVPH nucleic acid molecule comprising the DNA sequence of SEQ ID NOs:1-3 and isolated SVPH nucleic acid molecules encoding the amino acid sequence of SEQ ID NOs:4-6, as well as nucleic acid molecules complementary to these sequences. Further studies have revealed the full-length nucleotide sequences of three alternatively spliced SVPH-1 clones (SEQ ID NOs:7-9) and two alternatively spliced SVPH 4 clones (SEQ ID NOs:10-11). Thus, further embodiments of the invention are directed to an isolated SVPH nucleic acid molecule comprising the DNA sequence of SEQ ID NOs:7-11 and isolated SVPH nucleic acid molecules encoding the amino acid sequence of SEQ ID NOs:12-16, as well as nucleic acid molecules complementary to these sequences. Both single-stranded and double-stranded RNA and DNA nucleic acid molecules are encompassed by the invention, as well as nucleic acid molecules that hybridize to a denatured, double-stranded DNA comprising all or a portion of SEQ ID NOs:1-3 and 7-11. Also encompassed are isolated nucleic acid molecules that are derived by *in vitro* mutagenesis of nucleic acid molecules comprising sequences of SEQ ID NOs:1-3 and 7-11, that are degenerate from nucleic acid molecules comprising sequences of

nucleic acid molecules and host cells stably or transiently transformed or transfected with these vectors.

In addition, the invention encompasses methods of using the nucleic acids noted above to identify nucleic acids encoding proteins having metalloproteinase-disintegrin activities; to identify human chromosome number 1 or 4; to map genes on human chromosome number 1or 4; to identify genes associated with certain diseases, syndromes, or other human conditions associated with human chromosome number 1 or 4; and to study proteinases and their activities on cell/cell interactions as well as proteinase activity on the immune system.

The invention also encompasses the use of sense or antisense oligonucleotides from the nucleic acid of SEQ ID NOs:1-3 and 7-11 to inhibit the expression of the polynucleotides encoded by the SVPH-1, SVPH-3, or SVPH-4 genes.

The invention also encompasses isolated polypeptides and fragments thereof encoded by these nucleic acid molecules including soluble polypeptide portions of SEQ ID Nos:4-6 and 12-16. The invention further encompasses methods for the production of these polypeptides, including culturing a host cell under conditions promoting expression and recovering the polypeptide from the culture medium. Especially, the expression of these polypeptides in bacteria, yeast, plant, insect, and animal cells is encompassed by the invention.

In general, the polypeptides of the invention can be used to study the cell/cell and cell/matrix interactions involved in cellular processes (including cell fusion as in sperm/egg interactions, cell recognition and binding) as well as those involved in the immune system. In addition, these polypeptides can be used to identify other proteins associated with SVPH family members, ADAMs family members, and other metalloproteinases.

In addition, the invention includes assays utilizing these polypeptides to screen for potential inhibitors of activity associated with polypeptide counter-structure molecules, and methods of using these polypeptides as therapeutic agents for the treatment of diseases mediated by SVPH polypeptide counter-structure molecules.

The invention further provides a method for using these polypeptides as molecular weight markers that allow the estimation of the molecular weight of a

protein or a fragmented protein, as well as a method for the visualization of the molecular weight markers of the invention thereof using electrophoresis. The invention further encompasses methods for using the polypeptides of the invention as markers for determining the isoelectric point of an unknown protein, as well as controls for establishing the extent of fragmentation of a protein. Further encompassed by this invention are kits to aid in these determinations.

Isolated polyclonal or monoclonal antibodies that bind to these polypeptides are also encompassed by the invention, in addition the use of these antibodies to aid in purifying the SVPH polypeptide.

Further encompassed by this invention is the use of the SVPH nucleic acid sequences, predicted amino acid sequences of the polypeptide or fragments thereof, or a combination of the predicted amino acid sequences of the polypeptide and fragments thereof for use in searching an electronic database to aid in the identification of sample nucleic acids and/or proteins.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts a Northern blot hybridization showing the tissue specificity of SVPH-1 and SVPH-4.

Figure 2 depicts a phylogenetic tree of metalloproteinase-disintegrins. Branches marked with heavy lines indicate ADAM family members with a consensus zinc-binding motif (**HEXXHXXGXXHD**) (SEQ ID NO:31). The arrow indicates the probable zinc-binding motif containing common ancestor. Lineages in which the zinc-binding site was subsequently lost are denoted with an 'X'. Species abbreviations: Mm, *Mus musculus*; Rn, *Rattus norvegicus*; Hs, *Homo sapiens*; Mf, *Macaca fascicularis*; Oc, *Oryctolagus cuniculus*; Cc, *Cavia cobaya*, Cp, *Cavia porcellus*; So, *Saguinus oedipus*; Pp, *Pongo pygmaeus*; Bt, Bos *taurus*.

DETAILED DESCRIPTION OF THE INVENTION

Name: SVPH-1

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  1  ATTTTTGATA CCACAGTGAC CAACACGGTC ACCTAAGGTG TTCAATTCTT
 51  TGTAGCAAGT CTCACTTGCA GTATTTGCGC CTGCACCAAA AATCCTCCTA
101  CACTGTTCAN TTGCGGTCAT GACANGCTC       (SEQ ID NO:1)
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Name: SVPH-3

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  1 TTTTTGAGTA AGAATAGGTC ATGTTTTAGT AAAACTTCCA AAAGAACAAA
 51 ACAGATTCTT CAACCCAGGA GGACATGTGA GTCACAATAC CCTTTAATCC
101 ACAGGTTGGC TCCTTGGTTT CTGGAACTTT CTGCCTCCTG TAAACGATGT
151 GCGGGTGGTA CCCTCCCTCA ACCAGTGGAT GCTTCTTCAC GGGTTCAATG
201 AAAAAGTCTC CATGTGGTAG TTGGAAAAAT CCAGTCAGTC CATGGCAGGC
251 ACTGAGGGCT GCCGTCCCAA CTCTGGTGCC CTGCTGTAGA ACCGTGCCAC
301 TGAGATGGCA GAGGGGGGCA GAGGAAGCCA TCATCTTAAC ATGGGAGAGG
351 TTCCCATATC TCTTCTCCAT GATGTAGCTA TTGGAAAGAA ATCCTTCATT
401 GACCGTCAAG TTAAAAAACA GGTCCTTCTC CTCGTGAGAA ATTCTGTAGT
451 ACACCCAGTC CTCTGAGCC (SEQ ID NO:2)
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Name: SVPH-4

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   1 CACGAGGATT TATATCTTCA AAGAAAATAT AATGATGCTC TTGCATGGTC
  51 GTTTGGAAAA GTGTGTTCTC TAGAATATGC TGGATCAGTG AGTACTTTAC
 101 TAGATACAAA TATCCTTGCC CCTGCTACCT GGTCTGCTCA TGAGCTGGGT
 151 CATGCTGTAG GAATGTCACA TGATGAACAA TACTGCCAAT GTAGGGGTAG
 201 GCCTAATTGC ATCATGGGCT CAGGACGCAC TGGGTTTAGC AATTGCAGTT
 251 ATATCTCTTT TTTTAAACAT ATCTCTTCGG GAGCAACATG TCTAAATAAT
 301 ATCCCAGGAC TAGGTTATGT GCTTAAGAGA TGTGGAAACA AAATTGTGGA
 351 GGACAATGAG GAATGTGATT GTGGTTCCAC AGAGGAGTGT CAGAAAGATC
 401 GGTGTTGCCA ATCAAATTGT AAGTTGCAAC CAGGTGCCAA CTGTAGCATT
 451 GGACTTTGCT GTCATGATTG TCGGTTTCGT CCATCTGGAT ACGTGTGTAG
 501 GCAGGAAGGA AATGAATGTG ACCTTGCAGA GTACTGCGAC GGGAATTCAA
 551 GTTCCTGCCC AAATGACGTT TATAAGCAGG ATGGAACCCC TTGCAAGTAT
 601 GAAGGCCGTT GTTTCAGGAA GGGGTGCAGA TCCAGATATA TGCAGTGCCA
 651 AAGCATTTTT GGACCTGATG CCATGGAGGC TCCTAGTGAG TGCTATGATG
 701 CAGTTAACTT AATAGGTGAT CAATTTGGTA ACTGTGAGAT TACAGGAATT
 751 CGAAATTTTA AAAAGTGTGA AAGTGCAAAT TCAATATGTG GCAGGCTACA
 801 GTGTATAAAT GTTGAAACCA TCCCTGATTT GCCAGAGCAT ACGACTATAA
 851 TTTCTACTCA TTTACAGGCA GAAAATCTCA TGTGCTGGGG CACAGGCTAT
 901 CATCTATCCA TGAAACCCAT GGGAATACCT GACCTAGGTA TGATAAATGA
 951 TGGCACCTCC TGTGGAGAAG GCCGGGTATG TTTTAAAAAA AATTGCGTCA
1001 ATAGCTCAGT CCTGCAGTTT GACTGTTTGC CTGAGAAATG CAATACCCGG
1051 GGTGTTTGCA ACAACAGAAA AAACTGCCAC TGCATGTATG GGTGGGCACC
1101 TCCATTCTGT GAGGAAGTGG GGTATGGAGG AAGCATTGAC AGTGGGCCTC
1151 CAGGACTGCT CAGAGGGGCG ATTCCCTCGT CAATTTGGGT TGTGTCCATC
1201 ATAATGTTTC GCCTTATTTT ATTAATCCTT TCAGTGGTTT TTGTGTTTTT
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1451 TTGAAAGTAA AGAATGCAAA AAAAGATTCT TAAAAAACA AAAAAAGTAA
     SEQ ID NO:3
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Name: SVPH-1a

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   1  ATGAAGATGT TACTCCTGCT GCATTGCCTT GGGGTGTTTC TGTCCTGTTC
  51  TGGACACATC CAGGATGAGC ACCCCCAATA TCACAGCCCT CCGGATGTGG
 101  TGATTCCTGT GAGCATAACT GGCACCACCA GAGGCATGAC ACCTCCAGGC
 151  TGGCTCTCCT ATATCCTGCC CTTTGGAGGC CAGAAACACA TTATCCACAT
 201  AAAGGTCAAG AAGCTTTTGT TTTCCAAACA CCTCCCTGTG TTCACCTACA
 251  CAGACCAGGG TGCTATCCTT GAGGACCAGC CATTTGTCCA GAATAACTGC
 301  TACTATCATG GTTATGTGGA AGGGGACCCA GAATCCCTGG TTTCCCTCAG
 351  TACCTGTTTT GGGGGTTTTC AAGGAATATT ACAGATAAAT GACTTTGCTT
 401  ATGAAATCAA GCCCCTAGCA TTTTCTACCA CGTTTGAACA TCTGGTATAC
 451  AAGATGGACA GTGAGGAGAA ACAATTTTCA ACCATGAGAT CCGGATTTAT
 501  GCAAAATGAA ATAACATGCC GAATGGAATT TGAAGAAATT GATAATTCCA
 551  CTCAGAAGCA AAGTTCTTAT GTGGGCTGGT GGATCCATTT TAGGATTGTT
 601  GAAATTGTAG TCGTCATTGA TAATTATCTG TACATTCGTT ATGAAAGGAA
 651  CGACTCAAAG TTGCTGGAGG ATCTATATGT TATTGTTAAT ATAGTGGATT
 701  CCATTTTGGA TGTCATTGGT GTTAAGGTGT TATTATTTGG TTTGGAGATC
 751  TCCACCAATA AAAACCTCAT TCTACTACAT GATGTAAGGA AATCTGTGCA
 801  CCTGTATTGC AAGTGGAAGT CGGAGAACAT TACGCCCCGG ATGCAACATG
 851  ACACCTCACA TCTTTTCACA ACTCTAGGAT TAAGAGGGTT AAGTGGCATA
 901  GGAGCTTTTA GAGGAATGTG TACACCACAC CGTAGTTGTG CAATTGTTAC
 951  TTTCATGAAC AAAACTTTGG GCACTTTTTC AATTGCAGTG GCTCATCATC
1001  TAGGTCATAA TTTGGGCATG AACCATGATG AGGATACATG TCGTTGTTCA
1051  CAACCTAGAT GCATAATGCA TGAAGGCAAC CCACCAATAA CTAAATTTAG
1101  CAATTGTAGT TATGGTGATT TTTGGGAATA TACTGTAGAG AGGACAAAGT
1151  GTTTGCTTGA AACAGTACAC ACAAAGGACA TCTTTAATGT GAAGCGCTGT
1201  GGGAATGGTG TTGTTGAAGA AGGAGAAGAG TGTGACTGTG GACCTTTAAA
1251  GCATTGTGCA AAAGATCCCT GCTGTCTGTC AAATTGCACT CTGACTGATG
1301  GTTCTACTTG TGCTTTTGGG CTTTGTTGCA AAGACTGCAA GTTCCTACCA
1351  TCAGGGAAAG TGTGTAGAAA GGAGGTCAAT GAATGTGATC TTCCAGAGTG
1401  GTGCAATGGT ACTTCCCATA AGTGCCCAGA TGACTTTTAT GTGGAAGATG
1451  GAATTCCCTG TAAGGAGAGG GGCTACTGCT ATGAAAAGAG CTGTCATGAC
1501  CGCAATGAAC AGTGTAGGAG GATTTTTGGT GCAGGCGCAA ATACTGCAAG
1551  TGAGACTTGC TACAAAGAAT TGAACACCTT AGGTGACCGT GTTGGTCACT
1601  GTGGTATCAA AAATGCTACA TATATAAAGT GTAATATCTC AGATGTCCAG
1651  TGTGGAAGAA TTCAGTGTGA GAATGTGACA GAAATTCCCA ATATGAGTGA
1701  TCATACTACT GTGCATTGGG CTCGCTTCAA TGACATAATG TGCTGGAGTA
1751  CTGATTACCA TTTGGGGATG AAGGGACCTG ATATTGGTGA AGTGAAAGAT
1801  GGAACAGAGT GTGGGATAGA TCATATATGC ATCCACAGGC ACTGTGTCCA
1851  TATAACCATC TTGAATAGTA ATTGCTCACC TGCATTTTGT AACAAGAGGG
1901  GCATCTGCAA CAATAAACAT CACTGCCATT GCAATTATCT GTGGGACCCT
1951  CCCAACTGCC TGATAAAAGG CTATGGAGGT AGTGTTGACA GTGGCCCACC
2001  CCCTAAGAGA AAGAAGAAAA AGAAGTTCTG TTATCTGTGT ATATTGTTGC
2051  TTATTGTTTT GTTTATTTTA TTATGTTGTC TTTATCGACT TTGTAAAAAA
2101  AGTAAACCAA TAAAAAAGCA GCAAGATGTT CAAACTCCAT CTGCAAAAGA
2151  AGAGGAAAAA ATTCAGCGTC GACCTCATGA GTTACCTCCC CAGAGTCAAC
2201  CTTGGGTGAT GCCTTCCCAG AGTCAACCTC CTGTGACACC CTCCCAGAGG
2251  CAACCTCAGT TGATGCCTTC CCAGAGTCAA CCTCCTGTGA CGCCCTCCTA
2301  G  (SEQ ID NO:7)
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Name: SVPH-1b

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      ATGAAGATGT TACTCCTGCT GCATTGCCTT GGGGTGTTTC TGTCCTGTTC
  51  TGGACACATC CAGGATGAGC ACCCCCAATA TCACAGCCCT CCGGATGTGG
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 101  TGATTCCTGT GAGGATAACT GGCACCACCA GAGGCATGAC ACCTCCAGGC
 151  TGGCTCTCCT ATATCCTGCC CTTTGGAGGC CAGAAACACA TTATCCACAT
 201  AAAGGTCAAG AAGCTTTTGT TTTCCAAACA CCTCCCTGTG TTCACCTACA
 251  CAGACCAGGG TGCTATCCTT GAGGACCAGC CATTTGTCCA GAATAACTGC
 301  TACTATCATG GTTATGTGGA AGGGGACCCA GAATCCCTGG TTTCCCTCAG
 351  TACCTGTTTT GGGGGTTTTC AAGGAATATT ACAGATAAAT GACTTTGCTT
 401  ATGAAATCAA GCCCCTAGCA TTTTCTACCA CGTTTGAACA TCTGGTATAC
 451  AAGATGGACA GTGAGGAGAA ACAATTTTCA ACCATGAGAT CCGGATTTAT
 501  GCAAAATGAA ATAACATGCC GAATGGAATT TGAAGAAATT GATAATTCCA
 551  CTCAGAAGCA AAGTTCTTAT GTGGGCTGGT GGATCCATTT TAGGATTGTT
 601  GAAATTGTAG TCGTCATTGA TAATTATCTG TACATTCGTT ATGAAAGGAA
 651  CGACTCAAAG TTGCTGGAGG ATCTATATGT TATTGTTAAT ATAGTGGATT
 701  CCATTTTGGA TGTCATTGGT GTTAAGGTGT TATTATTTGG TTTGGAGATC
 751  TGGACCAATA AAAACCTCAT TGTAGTAGAT GATGTAAGGA AATCTGTGCA
 801  CCTGTATTGC AAGTGGAAGT CGGAGAACAT TACGCCCCGG ATGCAACATG
 851  ACACCTCACA TCTTTTCACA ACTCTAGGAT TAAGAGGGTT AAGTGGCATA
 901  GGAGCTTTTA GAGGAATGTG TACACCACAC CGTAGTTGTG CAATTGTTAC
 951  TTTCATGAAC AAAACTTTGG GCACTTTTTC AATTGCAGTG GCTCATCATC
1001  TAGGTCATAA TTTGGGCATG AACCATGATG AGGATACATG TCGTTGTTCA
1051  CAACCTAGAT GCATAATGCA TGAAGGCAAC CCACCAATAA CTAAATTTAG
1101  CAATTGTAGT TATGGTGATT TTTGGGAATA TACTGTAGAG AGGACAAAGT
1151  GTTTGCTTGA AACAGTACAC ACAAAGGACA TCTTTAATGT GAAGCGCTGT
1201  GGGAATGGTG TTGTTGAAGA AGGAGAAGAG TGTGACTGTG GACCTTTAAA
1251  GCATTGTGCA AAAGATCCCT GCTGTCTGTC AAATTGCACT CTGACTGATG
1301  GTTCTACTTG TGCTTTTGGG CTTTGTTGCA AAGACTGCAA GTTCCTACCA
1351  TCAGGGAAAG TGTGTAGAAA GGAGGTCAAT GAATGTGATC TTCCAGAGTG
1401  GTGCAATGGT ACTTCCCATA AGTGCCCAGA TGACTTTTAT GTGGAAGATG
1451  GAATTCCCTG TAAGGAGAGG GGCTACTGCT ATGAAAAGAG CTGTCATGAC
1501  CGCAATGAAC AGTGTAGGAG GATTTTTGGT GCAGGCGCAA ATACTGCAAG
1551  TGAGACTTGC TACAAAGAAT TGAACACCTT AGGTGACCGT GTTGGTCACT
1601  GTGGTATCAA AAATGCTACA TATATAAAGT GTAATATCTC AGATGTCCAG
1651  TGTGGAAGAA TTCAGTGTGA GAATGTGACA GAAATTCCCA ATATGAGTGA
1701  TCATACTACT GTGCATTGGG CTCGCTTCAA TGACATAATG TGCTGGAGTA
1751  CTGATTACCA TTTGGGGATG AAGGGACCTG ATATTGGTGA AGTGAAAGAT
1801  GGAACAGAGT GTGGGATAGA TCATATATGC ATCCACAGGC ACTGTGTCCA
1851  TATAACCATC TTGAATAGTA ATTGCTCACC TGCATTTTGT AACAAGAGGG
1901  GCATCTGCAA CAATAAACAT CACTGCCATT GCAATTATCT GTGGGACCCT
1951  CCCAACTGCC TGATAAAAGG CTATGGAGGT AGTGTTGACA GTGGTCCACC
2001  CCCTAAGAGA AAGAAGAAAA AGAAGTTCTG TTATCTGTGT ATATTGTTGC
2051  TTATTGTTTT GTTTATTTTA TTATGTTGTC TTTATCGACT TTGTAAAAAA
2101  AGTAAACCAA TAAAAAAGCA GCAAGATGTT CAAACTCCAT CTGCAAAAGA
2151  AGAGGAAAAA ATTCAGCGTC GACCTCATGA GTTACCTCCC CAGAGTCAAC
2201  CTTGGGTGAT GCCTTCCCAG AGTCAACCTC CTGTGACGCC TTCCCAGAGT
2251  CATCCTCAGG TGATGCCTTC CCAGAGTCAA CCTCCTCAAA ATTTATTCCT
2301  GTTCAGCTTC TCAATCAGTG ACTGTGTGCT AAATTTTAGG CTACTGTATC
2351  TTCAGGCCAC CTGA  (SEQ ID NO:8)
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Name: SVPH-1c

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 101  TGGCTCTCCT ATATCCTGCC CTTTGGAGGC CAGAAACACA TTATCCACAT
 201  AAAGGTCAAG AAGCTTTTGT TTTCCAAACA CCTCCCTGTG TTCACCTACA
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 251  CAGACCAGGG TGCTATCCTT GAGGACCAGC CATTTGTCCA GAATAACTGC
 301  TACTATCATG GTTATGTGGA AGGGGACCCA GAATCCCTGG TTTCCCTCAG
 351  TACCTGTTTT GGGGGTTTTC AAGGAATATT ACAGATAAAT GACTTTGCTT
 401  ATGAAATCAA GCCCCTAGCA TTTTCTACCA CGTTTGAACA TCTGGTATAC
 451  AAGATGGACA GTGAGGAGAA ACAATTTTCA ACCATGAGAT CCGGATTTAT
 501  GCAAAATGAA ATAACATGCC GAATGGAATT TGAAGAAATT GATAATTCCA
 551  CTCAGAAGCA AAGTTCTTAT GTGGGCTGGT GGATCCATTT TAGGATTGTT
 601  GAAATTGTAG TCGTCATTGA TAATTATCTG TACATTCGTT ATGAAAGGAA
 651  CGACTCAAAG TTGCTGGAGG ATCTATATGT TATTGTTAAT ATAGTGGATT
 701  CCATTTTGGA TGTCATTGGT GTTAAGGTGT TATTATTTGG TTTGGAGATC
 751  TGGACCAATA AAAACCTCAT TGTAGTAGAT GATGTAAGGA AATCTGTGCA
 801  CCTGTATTGC AAGTGGAAGT CGGAGAACAT TACGCCCCGG ATGCAACATG
 851  ACACCTCACA TCTTTTCACA ACTCTAGGAT TAAGAGGGTT AAGTGGCATA
 901  GGAGCTTTTA GAGGAATGTG TACACCACAC CGTAGTTGTG CAATTGTTAC
 951  TTTCATGAAC AAAACTTTGG GCACTTTTTC AATTGCAGTG GCTCATCATC
1001  TAGGTCATAA TTTGGGCATG AACCATGATG AGGATACATG TCGTTGTTCA
1051  CAACCTAGAT GCATAATGCA TGAAGGCAAC CCACCAATAA CTAAATTTAG
1101  CAATTGTAGT TATGGTGATT TTTGGGAATA TACTGTAGAG AGGACAAAGT
1151  GTTTGCTTGA AACAGTACAC ACAAAGGACA TCTTTAATGT GAAGCGCTGT
1201  GGGAATGGTG TTGTTGAAGA AGGAGAAGAG TGTGACTGTG GACCTTTAAA
1251  GCATTGTGCA AAAGATCCCT GCTGTCTGTC AAATTGCACT CTGACTGATG
1301  GTTCTACTTG TGCTTTTGGG CTTTGTTGCA AAGACTGCAA GTTCCTACCA
1351  TCAGGGAAAG TGTGTAGAAA GGAGGTCAAT GAATGTGATC TTCCAGAGTG
1401  GTGCAATGGT ACTTCCCATA AGTGCCCAGA TGACTTTTAT GTGGAAGATG
1451  GAATTCCCTG TAAGGAGAGG GGCTACTGCT ATGAAAAGAG CTGTCATGAC
1501  CGCAATGAAC AGTGTAGGAG GATTTTTGGT GCAGGCGCAA ATACTGCAAG
1551  TGAGACTTGC TACAAAGAAT TGAACACCTT AGGTGACCGT GTTGGTCACT
1601  GTGGTATCAA AAATGCTACA TATATAAAGT GTAATATCTC AGATGTCCAG
1651  TGTGGAAGAA TTCAGTGTGA GAATGTGACA GAAATTCCCA ATATGAGTGA
1701  TCATACTACT GTGCATTGGG CTCGCTTCAA TGACATAATG TGCTGGAGTA
1751  CTGATTACCA TTTGGGGATG AAGGGACCTG ATATTGGTGA AGTGAAAGAT
1801  GGAACAGAGT GTGGGATAGA TCATATATGC ATCCACAGGC ACTGTGTCCA
1851  TATAACCATC TTGAATAGTA ATTGCTCACC TGCATTTTGT AACAAGAGGG
1901  GCATCTGCAA CAATAAACAT CACTGCCATT GCAATTATCT GTGGGACCCT
1951  CCCAACTGCC TGATAAAAGG CTATGGAGGT AGTGTTGACA GTGGCCCACC
2001  CCCTAAGAGA AAGAAGAAAA AGAAGTTCTG TTATCTGTGT ATATTGTTGC
2051  TTATTGTTTT GTTTATTTTA TTATGTTGTC TTTATCGACT TTGTAAAAAA
2101  AGTAAACCAA TAAAAAAGCA GCAAGATGTT CAAACTCCAT CTGCAAAAGA
2151  AGAGGAAAAA ATTCAGCGTC GACCTCATGA GTTACCTCCC CAGAGTCAAC
2201  CTTGGGTGAT GCCTTCCCAG AGTCAACCTC CTGTGACGCC TTCCCAGAGT
2251  CATCCTCGGG TGATGCCTTC TCAGAGTCAA CCTCCTGTGA TGCCTTCCCA
2301  GAGTCATCCT CAGTTGACGC CTTCCCAGAG TCAACCTCCT GTGATGCCTT
2351  CCCAGAGTCA TCCTCAGTTG ACGCCTTCCC AGAGTCAACC TCCTGTGACA
2401  CCCTCCCAGA GGCAACCTCA GTTGATGCCT TCCCAGAGTC AACCTCCTGT
2451  GACGCCCTCC TAG  (SEQ ID NO:9)
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Name: SVPH-4a

```
 1  TTCTGGGGAA AGGTCAAGGA TATTGGTGAA TACTTGTCCT ACCTAATTGCA
51  GTTAAAAGGA AAGAAGCAC TCCTCCATTT TTGGCCCAAG AGAGTTCTGT
251 TGCCCCGACA TCTGCGCGTT TTCTGCTTCA CAGAACATCC CTAACTTCTT
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 301  GAGGATCATC CTTACATACC AAAGGACTGC AACTACATGG GCTCCGTGAA
 351  AGAGTCTCTG GACTCTAAAG CTACTATAAG CACATGCATG GGGGGTCTCC
 401  GAGGTGTATT TAACATTGAT GCCAAACATT ACCAAATTGA GCCCCTCAAG
 451  GCCTCTCCCA GTTTTGAACA TGTCGTCTAT CTCCTGAAGA AAGAGCAGTT
 501  TGGGAATCAG GTTTGTGGCT TAAGTGATGA TGAAATAGAA TGGCAGATGG
 551  CCCCTTATGA GAATAAGGCG AGGCTAAGGG ACTTTCCTGG ATCCTATAAA
 601  CACCCAAAGT ACTTGGAATT GATCCTACTC TTTGATCAAA GTAGGTATAG
 651  GTTTGTGAAC AACAATCTTT CTCAAGTCAT ACATGATGCC ATTCTTTTGA
 701  CTGGGATTAT GGACACCTAC TTTCAAGATG TTCGTATGAG GATACACTTA
 751  AAGGCTCTTG AAGTATGGAC AGATTTTAAC AAAATACGCG TTGGATATCC
 801  AGAGTTAGCT GAAGTTTTAG GCAGATTTGT AATATATAAA AAAAGTGTAT
 851  TAAATGCTCG CCTGTCATCA GATTGGGCAC ATTTATATCT TCAAAGAAAA
 901  TATAATGATG CTCTTGCATG GTCGTTTGGA AAAGTGTGTT CTCTAGAATA
 951  TGCTGGATCA GTGAGTACTT TACTAGATAC AAATATCCTT GCCCCTGCTA
1001  CCTGGTCTGC TCATGAGCTG GGTCATGCTG TAGGAATGTC ACATGATGAA
1051  CAATACTGCC AATGTAGGGG TAGGCCTAAT TGCATCATGG GCTCAGGACG
1101  CACTGGGTTT AGCAATTGCA GTTATATCTC TTTTTTTAAA CATATCTCTT
1151  CGGGAGCAAC ATGTCTAAAT AATATCCCAG GACTAGGTTA TGTGCTTAAG
1201  AGATGTGGAA ACAAAATTGT GGAGGACAAT GAGGAATGTG ATTGTGGTTC
1251  CACAGAGGAG TGTCAGAAAG ATCGGTGTTG CCAATCAAAT TGTAAGTTGC
1301  AACCAGGTGC CAACTGTAGC ATTGGACTTT GCTGTCATGA TTGTCGGTTT
1351  CGTCCATCTG GATACGTGTG TAGGCAGGAA GGAAATGAAT GTGACCTTGC
1401  AGAGTACTGC GACGGGAATT CAAGTTCCTG CCCAAATGAC GTTTATAAGC
1451  AGGATGGAAC CCCTTGCAAG TATGAAGGCC GTTGTTTCAG GAAGGGGTGC
1501  AGATCCAGAT ATATGCAGTG CCAAAGCATT TTTGGACCTG ATGCCATGGA
1551  GGCTCCTAGT GAGTGCTATG ATGCAGTTAA CTTAATAGGT GATCAATTTG
1601  GTAACTGTGA GATTACAGGA ATTCGAAATT TTAAAAAGTG TGAAAGTGCA
1651  AATTCAATAT GTGGCAGGCT ACAGTGTATA AATGTTGAAA CCATCCCTGA
1701  TTTGCCAGAG CATACGACTA TAATTTCTAC TCATTTACAG GCAGAAAATC
1751  TCATGTGCTG GGGCACAGGC TATCATCTAT CCATGAAACC CATGGGAATA
1801  CCTGACCTAG GTATGATAAA TGATGGCACC TCCTGTGGAG AAGGCCGGGT
1851  ATGTTTTAAA AAAAATTGCG TCAATAGCTC AGTCCTGCAG TTTGACTGTT
1901  TGCCTGAGAA ATGCAATACC CGGGGTGTTT GCAACAACAG AAAAAACTGC
1951  CACTGCATGT ATGGGTGGGC ACCTCCATTC TGTGAGGAAG TGGGGTATGG
2001  AGGAAGCATT GACAGTGGGC CTCCAGGACT GCTCAGAGGG GCGATTCCCT
2051  CGTCAATTTG GGTTGTGTCC ATCATAATGT TTCGCCTTAT TTTATTAATC
2101  CTTTCAGTGG TTTTTGTGTT TTTCCGGCAA GTGATAGGAA ACCACTTAAA
2151  ACCCAAACAG GAAAAAATGC CACTATCCAA AGCAAAAACT GAACAGGAAG
2201  AATCTAAAAC AAAAACTGTA CAGGAAGAAT CTAAAACAAA AACTGGACAG
2251  GAAGAATCTG AAGCAAAAAC TGGACAGGAA GAATCTAAAG CAAAAACTGG
2301  ACAGGAAGAA TCTAAAGCAA ACATTGAAAG TAAACGACCC AAAGCAAAGA
2351  GTGTCAAGAA ACAAAAAAAG TAA  (SEQ ID NO:10)
```

Name: SVPH-4b

```
   1  ATGAGGTCAG TGCAGATCTT CCTCTCCCAA TGCCGTTTGC TCCTTCTACT
  51  AGTTCCGACA ATGCTCCTTA AGTCTCTTGG CGAAGATGTA ATTTTTCACC
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```
   1  AAGAATCAT  CTTACATACC AAAGGACTGC AACTACATGG GCTCCGTGAA
 351  AGAGTCTCTG GACTCTAAAG CTACTATAAG CACATGCATG GGGGGTCTCC
 401  GAGGTGTATT TAACATTGAT GCCAAACATT ACCAAATTGA GCCCCTCAAG
```

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 451  GCCTCTCCCA GTTTTGAACA TGTCGTCTAT CTCCTGAAGA AAGAGCAGTT
 501  TGGGAATCAG GTTTGTGGCT TAAGTGATGA TGAAATAGAA TGGCAGATGG
 551  CCCCTTATGA GAATAAGGCG AGGCTAAGGG ACTTTCCTGG ATCCTATAAA
 601  CACCCAAAGT ACTTGGAATT GATCCTACTC TTTGATCAAA GTAGGTATAG
 651  GTTTGTGAAC AACAATCTTT CTCAAGTCAT ACATGATGCC ATTCTTTTGA
 701  CTGGGATTAT GGACACCTAC TTTCAAGATG TTCGTATGAG GATACACTTA
 751  AAGGCTCTTG AAGTATGGAC AGATTTTAAC AAAATACGCG TTGGATATCC
 801  AGAGTTAGCT GAAGTTTTAG GCAGATTTGT AATATATAAA AAAAGTGTAT
 851  TAAATGCTCG CCTGTCATCA GATTGGGCAC ATTTATATCT TCAAAGAAAA
 901  TATAATGATG CTCTTGCATG GTCGTTTGGA AAAGTGTGTT CTCTAGAATA
 951  TGCTGGATCA GTGAGTACTT TACTAGATAC AAATATCCTT GCCCCTGCTA
1001  CCTGGCCTGC TCATGAGCTG GGTCATGCTG TAGGAATGTC ACATGATGAA
1051  CAATACTGCC AATGTAGGGG TAGGCTTAAT TGCATCATGG GCTCAGGACG
1101  CACTGGGTTT AGCAATTGCA GTTATATCTC TTTTTTTAAA CATATCTCTT
1151  CGGGAGCAAC ATGTCTAAAT AATATCCCAG GACTAGGTTA TGTGCTTAAG
1201  AGATGTGGAA ACAAAATTGT GGAGGACAAT GAGGAATGTG ACTGTGGTTC
1251  CACAGAGGAG TGTCAGAAAG ATCGGTGTTG CCAATCAAAT TGTAAGTTGC
1301  AACCAGGTGC CAACTGTAGC ATTGGACTTT GCTGTCATGA TTGTCGGTTT
1351  CGTCCATCTG GATACGTGTG TAGGCAGGAA GGAAATGAAT GTGACCTTGC
1401  AGAGTACTGC GACGGGAATT CAAGTTCCTG CCCAAATGAC GTTTATAAGC
1451  AGGATGGAAC CCCTTGCAAG TATGAAGGCC GTTGTTTCAG GAAGGGGTGC
1501  AGATCCAGAT ATATGCAGTG CCAAAGCATT TTTGGACCTG ATGCCATGGA
1551  GGCTCCTAGT GAGTGCTATG ATGCAGTTAA CTTAATAGGT GATCAATTTG
1601  GTAACTGTGA GATTACAGGA ATTCGAAATT TTAAAAAGTG TGAAAGTGCA
1651  AATTCAATAT GTGGCAGGCT ACAGTGTATA AATGTTGAAA CCATCCCTGA
1701  TTTGCCAGAG CATACGACTA TAATTTCTAC TCATTTACAG GCAGAAAATC
1751  TCATGTGCTG GGGCACAGGC TATCATCTAT CCATGAAACC CATGGGAATA
1801  CCTGACCTAG GTATGATAAA TGATGGCACC TCCTGTGGAG AAGGCCGGGT
1851  ATGTTTTAAA AAAAATTGCG TCAATAGCTC AGTCCTGCAG TTTGACTGTT
1901  TGCCTGAGAA ATGCAATACC CGGGGTGTTT GCAACAACAG AAAAAACTGC
1951  CACTGCATGT ATGGGTGGGC ACCTCCATTC TGTGAGGAAG TGGGGTATGG
2001  AGGAAGCATT GACAGTGGGC CTCCAGGACT GCTCAGAGGG GCGATTCCCT
2051  CGTCAATTTG GGTTGTGTCC ATCATAATGT TTCGCCTTAT TTTATTAATC
2101  CTTTCAGTGG TTTTTGTGTT TTTCCGGCAA GTGATAGGAA ACCACTTAAA
2151  ACCCAAACAG GAAAAAATGC CACTATCCAA AGCAAAAACT GAACAGGAAG
2201  AATCTAAAAC AAAAACTGTA CAGGAAGAAT CTAAAACAAA AACTGGACAG
2251  GAAGAATCTG AAGCAAAAAC TGGACAGGAA GAATCTAAAG CAAACATTGA
2301  AAGTAAACGA CCCAAAGCAA AGAGTGTCAA GAAACAAAAA AAGTAA
      (SEQ ID NO:11)
```

The amino acid sequences of the polypeptides encoded by the nucleotide sequence of the invention includes:

Name: SVPH-1 (polypeptide)

Name: SVPH-3 (polypeptide)

```
  1 EDWVYYRISH EEKDLFFNLT VNEGFLSNSY IMEKRYGNLS HVKMMASSAP
 51 LCHLSGTVLQ QGTRVGTAAL SACHGLTGFF QLPHGDFFIE PVKKHPLVEG
101 GYHPHIVYRR QKVPETKEPT CGL (SEQ ID NO:5)
```

Name: SVPH-4 (polypeptide)

```
  1  HEDLYLQRKY NDALAWSFGK VCSLEYAGSV STLLDTNILA PATWSAHELG
 51  HAVGMSHDEQ YCQCRGRPNC IMGSGRTGFS NCSYISFFKH ISSGATCLNN
101  IPGLGYVLKR CGNKIVEDNE ECDCGSTEEC QKDRCCQSNC KLQPGANCSI
151  GLCCHDCRFR PSGYVCRQEG NECDLAEYCD GNSSSCPNDV YKQDGTPCKY
201  EGRCFRKGCR SRYMQCQSIF GPDAMEAPSE CYDAVNLIGD QFGNCEITGI
251  RNFKKCESAN SICGRLQCIN VETIPDLPEH TTIISTHLQA ENLMCWGTGY
301  HLSMKPMGIP DLGMINDGTS CGEGRVCFKK NCVNSSVLQF DCLPEKCNTR
351  GVCNNRKNCH CMYGWAPPFC EEVGYGGSID SGPPGLLRGA IPSSIWVVSI
401  IMFRLILLIL SVVFVFFRQV IGNHLKPKQE KMPLSKAKTE QEESKTKTVQ
451  EESKTKTGQE ESEAKTGQEE SKAKTGQEES KANIESKRPK AKSVKKQKK*
      (SEQ ID NO:6)
```

Name: SVPH-1a (polypeptide)

```
  1  MKMLLLLHCL GVFLSCSGHI QDEHPQYHSP PDVVIPVRIT GTTRGMTPPG
 51  WLSYILPFGG QKHIIHIKVK KLLFSKHLPV FTYTDQGAIL EDQPFVQNNC
101  YYHGYVEGDP ESLVSLSTCF GGFQGILQIN DFAYEIKPLA FSTTFEHLVY
151  KMDSEEKQFS TMRSGFMQNE ITCRMEFEEI DNSTQKQSSY VGWWIHFRIV
201  EIVVVIDNYL YIRYERNDSK LLEDLYVIVN IVDSILDVIG VKVLLFGLEI
251  WTNKNLIVVD DVRKSVHLYC KWKSENITPR MQHDTSHLFT TLGLRGLSGI
301  GAFRGMCTPH RSCAIVTFMN KTLGTFSIAV AHHLGHNLGM NHDEDTCRCS
351  QPRCIMHEGN PPITKFSNCS YGDFWEYTVE RTKCLLETVH TKDIFNVKRC
401  GNGVVEEGEE CDCGPLKHCA KDPCCLSNCT LTDGSTCAFG LCCKDCKFLP
451  SGKVCRKEVN ECDLPEWCNG TSHKCPDDFY VEDGIPCKER GYCYEKSCHD
501  RNEQCRRIFG AGANTASETC YKELNTLGDR VGHCGIKNAT YIKCNISDVQ
551  CGRIQCENVT EIPNMSDHTT VHWARFNDIM CWSTDYHLGM KGPDIGEVKD
601  GTECGIDHIC IHRHCVHITI LNSNCSPAFC NKRGICNNKH HCHCNYLWDP
651  PNCLIKGYGG SVDSGPPPKR KKKKKFCYLC ILLLIVLFIL LCCLYRLCKK
701  SKPIKKQQDV QTPSAKEEEK IQRRPHELPP QSQPWVMPSQ SQPPVTPSQP
751  QPQLMPSQSP PPVTPS*   (SEQ ID NO:12)
```

Name: SVPH-1b (polypeptide)

```
  1  MKMLLLLHCL GVFLSCSGHI QDEHPQYHSP PDVVIPVRIT GTTRGMTPPG
 51  WLSYILPFGG QKHIIHIKVK KLLFSKHLPV FTYTDQGAIL EDQPFVQNNC
101  YYHGYVEGDP ESLVSLSTCF GGFQGILQIN DFAYEIKPLA FSTTFEHLVY
151  KMDSEEKQFS TMRSGFMQNE ITCRMEFEEI DNSTQKQSSY VGWWIHFRI[illegible]

4[illegible]  [illegible]NGVVEEGEE [illegible] [illegible]CCLSNC[illegible] LTDGSTCAF[illegible] [illegible]CCKDCKFLP
451  [illegible] ECDLPEWCN[illegible] TSHKCPDDFY VEDGIPCKER GYCYEKSCH[illegible]
501  RNEQCRRIFG AGANTASET[illegible] [illegible]KELNTLGDR [illegible] YIKCNISD[illegible]
```

```
551  CGRIQCENVT EIPNMSDHTT VHWARFNDIM CWSTDYHLGM KGPDIGEVKD
601  GTECGIDHIC IHRHCVHITI LNSNCSPAFC NKRGICNNKH HCHCNYLWDP
651  PNCLIKGYGG SVDSGPPPKR KKKKKFCYLC ILLLIVLFIL LCCLYRLCKK
701  SKPIKKQQDV QTPSAKEEEK IQRRPHELPP QSQPWVMPSQ SQPPVTPSQS
751  HPQVMPSQSQ PPQNLFLFSF SISDCVLNFR LLYLQAT*  (SEQ ID NO:13)
```

Name: SVPH-1c (polypeptide)

```
   1  MKMLILLLHCL GVFLSCSGHI QDEHPQYHSP PDVVIPVRIT GTTRGMTPPG
  51  WLSYILPFGG QKHIIHIKVK KLLFSKHLPV FTYTDQGAIL EDQPFVQNNC
 101  YYHGYVEGDP ESLVSLSTCF GGFQGILQIN DFAYEIKPLA FSTTFEHLVY
 151  KMDSEEKQFS TMRSGFMQNE ITCRMEFEEI DNSTQKQSSY VGWWIHPRIV
 201  EIVVVIDNYL YIRYERNDSK LLEDLYVIVN IVDSILDVIG VKVLLFGLEI
 251  WTNKNLIVVD DVRKSVHLYC KWKSENITPR MQHDTSHLFT TLGLRGLSGI
 301  GAFRGMCTPH RSCAIVTFMN KTLGTFSIAV AHHLGHNLGM NHDEDTCRCS
 351  QPRCIMHEGN PPITKFSNCS YGDFWEYTVE RTKCLLETVH TKDIFNVKRC
 401  GNGVVEEGEE CDCGPLKHCA KDPCCLSNCT LTDGSTCAFG LCCKDCKFLP
 451  SGKVCRKEVN ECDLPEWCNG TSHRCPDDFY VEDGIPCKER GYCYEKSCHD
 501  RNEQCRRIFG AGANTASETC YKELNTLGDR VGHCGIKNAT YIKCNISDVQ
 551  CGRIQCENVT EIPNMSDHTT VHWARFNDIM CWSTDYHLGM KGPDIGEVKD
 601  GTECGIDHIC IHRHCVHITI LNSNCSPAFC NKRGICNNKH HCHCNYLWDP
 651  PNCLIKGYGG SVDSGPPPKR KKKKKFCYLC ILLLIVLFIL LCCLYRLCKK
 701  SKPIKKQQDV QTPSAKEEEK IQRRPHELPP QSQPWVMPSQ SQPPVTPSQS
 751  HPRVMPSQSQ PPVMPSQSHP QLTPSQSQPP VMPSQSHPQL TPSQSQPPVT
 801  PSQRQPQLMP SQSQPPVTPS *   (SEQ ID NO:14)
```

Name: SVPH-4a (polypeptide)

```
   1  MRSVQIFLSQ CRLLLLLVPT MLLKSLGEDV IFHPEGEFDS YEVTIPEKLS
  51  FRGEVQGVVS PVSYLLQLKG KKHVLHLWPK RLLLPRHLRV FSFTEHGELL
 101  EDHPYIPKDC NYMGSVKESL DSKATISTCM GGLRGVFNID AKHYQIEPLK
 151  ASPSFEHVVY LLKKEQFGNQ VCGLSDDEIE WQMAPYENKA RLRDFPGSYK
 201  HPKYLELILL FDQSRYRFVN NNLSQVIHDA ILLTGIMDTY FQDVRMRIHL
 251  KALEVWTDFN KIRVGYPELA EVLGRFVIYK KSVLNARLSS DWAHLYLQRK
 301  YNDALAWSFG KVCSLEYAGS VSTLLDTNIL APATWSAHEL GHAVGMSHDE
 351  QYCQCRGRPN CIMGSGRTGF SNCSYISFFK HISSGATCLN NIPGLGYVLK
 401  RCGNKIVEDN EECDCGSTEE CQKDRCCQSN CKLQPGANCS IGLCCHDCRF
 451  RPSGYVCRQE GNECDLAEYC DGNSSSCPND VYKQDGTPCK YEGRCFRKGC
 501  RSRYMQCQSI FGPDAMEAPS ECYDAVNLIC DQFGNCEITG IRNFKKCESA
 551  NSICGRLQCI NVETIPDLPE HTTIISTHLQ AENLMCWGTG YHLSMKPMGI
 601  PDLGMINDGT SCGEGRVCFK KNCVNSSVLQ FDCLPEKCNT PGVCNNRKNC
 651  HCMYGWAPPF CEEVGYGGSI DSGPPGLLRG AIPSSIWVVS IIMFRLILLI
 701  LSVVFVFFRQ VIGNHLKPKQ EFMPLSKAKT EQEESKTKTV QEESKTKTGQ
 751  EESEAKTGQE ESKAKTGQEE SKANIESKRP KAKSVKKQKK *
          (SEQ ID NO:15)
```

Name: SVPH-4b (polypeptide)

```
  1  EDHPYIPKDC NYMGSVKESL DSKATISTCM GGLRGVFNID AKHYQIEPLK
 51  ASPSFEHVVY LLKKEQFGNQ VCGLSDDEIE WQMAPYENKA RLRDFPGSYK
101  HPKYLELILL FDQSRYRFVN NNLSQVIHDA ILLTGIMDTY FQDVRMRIHL
```

```
251  KALEVWTDFN KIRVGYPELA EVLGRFVIYK KSVLNARLSS DWAHLYLQRK
301  YNDALAWSFG KVCSLEYAGS VSTLLDTNIL APATWPAHEL GHAVGMSHDE
351  QYCQCRGRLN CIMGSGRTGF SNCSYISFFK HISSGATCLN NIPGLGYVLK
401  RCGNKIVEDN EECDCGSTEE CQKDRCCQSN CKLQPGANCS IGLCCHDCRF
451  RPSGYVCRQE GNECDLAEYC DGNSSSCPND VYKQDGTPCK YEGRCFRKGC
501  RSRYMQCQSI FGPDAMEAPS ECYDAVNLIG DQFGNCEITG IRNFKKCESA
551  NSICGRLQCI NVETIPDLPE HTTIISTHLQ AENLMCWGTG YHLSMKPMGI
601  PDLGMINDGT SCGEGRVCFK KNCVNSSVLQ FDCLPEKCNT RGVCNNRKNC
651  HCMYGWAPPF CEEVGYGGSI DSGPPGLLRG AIPSSIWVVS IIMFRLILLI
701  LSVVFVFFRQ VIGNHLKPKQ EKMPLSKAKT EQEESKTKTV QEESKTKTGQ
751  EESEAKTGQE ESKANIESKR PKAKSVKKQK K*   (SEQ ID NO:16)
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The discovery of the nucleic acids of the invention enables the construction of expression vectors comprising nucleic acid sequences encoding polypeptides; host cells transfected or transformed with the expression vectors; isolated and purified biologically active polypeptides and fragments thereof; the use of the nucleic acids or oligonucleotides thereof as probes to identify nucleic acid encoding proteins having metalloproteinase-disintegrin activity; the use of the nucleic acids or oligonucleotides thereof to identify human chromosome number 1 or 4; the use of the nucleic acids or oligonucleotides thereof to map genes on human chromosome number 1 or 4; the use of the nucleic acid or oligonucleotides thereof to identify genes associated with certain diseases, syndromes or other human conditions associated with human chromosome number 1 or 4, including fetal hydantoin syndrome, diphenylhydantoin toxicity, and pheochromocytoma; the use of single-stranded sense or antisense oligonucleotides from the nucleic acids to inhibit expression of polynucleotide encoded by the SVPH-1, SVPH-3, or SVPH-4 gene; the use of such polypeptides and soluble fragments to function as a proteinase; the use of such polypeptides and fragmented peptides as molecular weight markers; the use of such polypeptides and fragmented peptides as controls for peptide fragmentation, and kits comprising these reagents; the use of such polypeptides and fragments thereof to generate antibodies; and the use of antibodies to purify SVPH polypeptides.

NUCLEIC ACID MOLECULES

In a particular embodiment, the invention relates to certain isolated nucleotide sequences that are free from contaminating endogenous material. A "nucleotide sequence" refers to a polynucleotide molecule in the form of a separate fragment or as a component of a larger nucleic acid construct. The nucleic acid molecule has been derived from DNA or RNA isolated at least once in substantially pure form and in a quantity or concentration enabling identification, manipulation, and recovery of its component nucleotide sequences by standard biochemical methods, such as those outlined in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989). Such sequences are preferably provided and/or constructed in the form of an open reading frame uninterrupted by internal non-translated sequences, or introns, that are typically present in eukaryotic genes. Sequences of non-translated DNA can be present 5' or 3' from an open reading frame, where the same do not interfere with manipulation or expression of the coding region.

Nucleic acid molecules of the invention include DNA in both single-stranded and double-stranded form, as well as the RNA complement thereof. DNA includes, for example, cDNA, genomic DNA, chemically synthesized DNA, DNA amplified by PCR, and combinations thereof. Genomic DNA may be isolated by conventional techniques, *e.g.*, using the cDNA of SEQ ID NOs:1-3 and 7-11, or a suitable fragment thereof, as a probe.

The DNA molecules of the invention include full length genes as well as polynucleotides and fragments thereof. The full length gene may include the N-terminal signal peptide. Other embodiments include DNA encoding a soluble form, *e.g.*, encoding the extracellular domain of the protein, either with or without the signal peptide.

The nucleic acids of the invention are preferentially derived from human

Preferred Sequences

Particularly preferred nucleotide sequences of the invention are SEQ ID NOs:1-3 and 7-11, as set forth above. The sequences of amino acids encoded by the DNA of SEQ ID NOs:1-3 and 7-11 are shown in SEQ ID NOs:4-6 and 12-16, respectively. In SEQ ID NO:1 "N" can represent any nucleotide. These sequences identify the SVPH polynucleotides as members of the metalloproteinase-disintegrin family. As noted above, proteins of this family are characterized by a pro-domain, a disintegrin domain, a metalloproteinase domain, a cysteine rich region, a transmembrane domain, and a cytoplasmic tail.

In particular, SVPH-1 (originally isolated from human testis) and SVPH-4 (originally isolated from human testis, fetal lung, and B-cells) both share homology to the cysteine rich region of the metalloproteinase-disintegrin family, and SVPH-3 (originally isolated from human fetus tissue) shares homology to the pro-domain of these family members. In addition, SVPH-4 polypeptide (SEQ ID NO:3) encodes a zinc binding motif (His 47 to Asp 58), a disintegrin domain (Leu 104 to Cys 179), and a cysteine rich region (Asp 180 to Arg 388).

SVPH-1a, SVPH-1b, and SVPH-1c represent the nucleotide sequences (SEQ ID NOs:7-9) of three alternatively spliced SVPH-1 clones with divergent cytoplasmic domains. These clones were isolated by screening a human testis library (Clonetech cat no. HL3024a) at 42°C and washing at 42°C in 2x SSC using four different oligonucleotides:

CACCTAAGGTGTTCAATTCTTTG (SEQ ID NO:17),

CAAATACTGCAAGTGAGACTTGC (SEQ ID NO:18),

TGCACAACTACGTGTGGTGTACCC (SEQ ID NO:19), and

GAGCCACTGCAATTGAAAAAGTGCCC (SEQ ID NO:20).

SVPH-4a and SVPH-4b represent the nucleotide sequences (SEQ ID NOs:10-11) of two alternatively spliced SVPH-4 clones with divergent cytoplasmic domains.

oligonucleotides:

[illegible] (SEQ ID NO:21).

CTTTCACGGAGCCCATGTAGTTGCAG (SEQ ID NO:22), and

TGAAGGAGAAAACGCGCAGATGTCGG (SEQ ID NO:23).

Additional Sequences

Due to the known degeneracy of the genetic code, wherein more than one codon can encode the same amino acid, a DNA sequence can vary from that shown in SEQ ID NOs:1-3 and 7-11, and still encode a polypeptide having the amino acid sequence of SEQ ID NOs:4-6 and 12-16, respectively. Such variant DNA sequences can result from silent mutations (*e.g.*, occurring during PCR amplification), or can be the product of deliberate mutagenesis of a native sequence.

The invention thus provides isolated DNA sequences encoding polypeptides of the invention, selected from: (a) DNA comprising the nucleotide sequence of SEQ ID NOs:1-3 and 7-11; (b) DNA encoding the polypeptides of SEQ ID NOs:4-6 and 12-16; (c) DNA capable of hybridization to a DNA of (a) or (b) under conditions of moderate stringency and which encodes polypeptides of the invention; (d) DNA capable of hybridization to a DNA of (a) or (b) under conditions of high stringency and which encodes polypeptides of the invention, and (e) DNA which is degenerate as a result of the genetic code to a DNA defined in (a), (b), (c), or (d) and which encode polypeptides of the invention. Of course, polypeptides encoded by such DNA sequences are encompassed by the invention.

As used herein, conditions of moderate stringency can be readily determined by those having ordinary skill in the art based on, for example, the length of the DNA. The basic conditions are set forth by Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2nd ed. Vol. 1, pp. 1.101-104, Cold Spring Harbor Laboratory Press, (1989), and include use of a prewashing solution for the nitrocellulose filters 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization conditions of about 50% formamide, 6X SSC at about 42°C (or other similar hybridization solution, such as

determined by the skilled artisan based on, for example, the length of the DNA. Generally, such conditions are defined as hybridization conditions as above, and with

washing at approximately 68°C, 0.2X SSC, 0.1% SDS. The skilled artisan will recognize that the temperature and wash solution salt concentration can be adjusted as necessary according to factors such as the length of the probe.

Also included as an embodiment of the invention is DNA encoding polypeptide fragments and polypeptides comprising inactivated N-glycosylation site(s), inactivated protease processing site(s), or conservative amino acid substitution(s), as described below.

In another embodiment, the nucleic acid molecules of the invention also comprise nucleotide sequences that are at least 80% identical to a native sequence. Also contemplated are embodiments in which a nucleic acid molecule comprises a sequence that is at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, or at least 99.9% identical to a native sequence.

The percent identity may be determined by visual inspection and mathematical calculation. Alternatively, the percent identity of two nucleic acid sequences can be determined by comparing sequence information using the GAP computer program, version 6.0 described by Devereux et al., *Nucl. Acids Res.*, 12:387 (1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res. 14*:6745 (1986), as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Other programs used by one skilled in the art of sequence comparison may also be used.

The invention also provides isolated nucleic acids useful in the production of polypeptides. Such polypeptides may be prepared by any of a number of conventional techniques. A DNA sequence encoding a [illegible] fragment [illegible] suitable leader or signal peptide. Alternatively, the desired fragment may be

chemically synthesized using known techniques. DNA fragments also may be produced by restriction endonuclease digestion of a full length cloned DNA sequence, and isolated by electrophoresis on agarose gels. If necessary, oligonucleotides that reconstruct the 5' or 3' terminus to a desired point may be ligated to a DNA fragment generated by restriction enzyme digestion. Such oligonucleotides may additionally contain a restriction endonuclease cleavage site upstream of the desired coding sequence, and position an initiation codon (ATG) at the N-terminus of the coding sequence.

The well-known polymerase chain reaction (PCR) procedure also may be employed to isolate and amplify a DNA sequence encoding a desired protein fragment. Oligonucleotides that define the desired termini of the DNA fragment are employed as 5' and 3' primers. The oligonucleotides may additionally contain recognition sites for restriction endonucleases, to facilitate insertion of the amplified DNA fragment into an expression vector. PCR techniques are described in Saiki et al., *Science* 239:487 (1988); *Recombinant DNA Methodology*, Wu et al., eds., Academic Press, Inc., San Diego, pp. 189-196 (1989); and *PCR Protocols: A Guide to Methods and Applications*, Innis et al., eds., Academic Press, Inc. (1990).

POLYPEPTIDES AND FRAGMENTS THEREOF

The invention encompasses polypeptides and fragments thereof in various forms, including those that are naturally occurring or produced through various techniques such as procedures involving recombinant DNA technology. Such forms include, but are not limited to, derivatives, variants, and oligomers, as well as fusion proteins or fragments thereof.

Polypeptides and Fragments Thereof

The polypeptides of the invention include the proteins encoded by the nucleic acid sequences set forth above. Particularly preferred polypeptides comprise the

region homologous to the metalloproteinase-disintegrin family, and the polypeptide of SEQ ID NO:5 includes a pro-domain homologous to the same family of proteins.

SVPH-1 (SEQ ID NO:4) has an N-terminal region having amino acids Met 1 to Asn 40. In SEQ ID NO:4 "X" can represent any amino acid. SVPH-3 (SEQ ID NO:5) has an N-terminal region having amino acids Asn 1 to Leu 23. SVPH-4 (SEQ ID NO:6) also includes an extracellular domain comprising amino acids His 1 to Arg 388, a transmembrane region comprising amino acids Gly 389 through Phe 417, and a C-terminal cytoplasmic domain comprising amino acids Arg 418 to Lys 499 and is believed to overlap with EST designated AA 782936.

The SVPH-1a polypeptide (SEQ ID NO:12), SVPH-1b polypeptide (SEQ ID NO:13), and SVPH-1c polypeptide (SEQ ID NO:14) each encodes a signal sequence (Met 1 to Ser 15), a pro-domain (Cys 16 to Ser 188), a catalytic domain (Ser 189 to Thr 388), a disintegrin domain (Val 389 to Gly 491), a cysteine rich region (Tyr 492 to Lys 675), and a transmembrane domain (Phe 676 to Cys 698). In addition, each of the SVPH-1a, SVPH-1b, and SVPH-1c polypeptides (SEQ ID NOs:12-14) encodes a cytoplasmic domain. Due to alternative splicing the cytoplasmic domain of each polypeptide is different. For SVPH-1a, SVPH-1b, and SVPH-1c the cytoplasmic domains are (Lys 699 to Ser 766), (Lys 699 to Thr 787), and (Lys 699 to Ser 820), respectively.

Similarly, the SVPH-4a polypeptide (SEQ ID NO:15) and SVPH-4b polypeptide (SEQ ID NO:16) each encodes a signal sequence (Met 1 to Gly 27), a pro-domain (Glu 28 to Arg 193), a catalytic domain (Asp 194 to Ile 392), a disintegrin domain (Pro 393 to Gly 493), a cysteine rich region (Arg 494 to Ser 685), and a transmembrane domain (Ile 686 to Gly 713). In addition, each of the SVPH-4a and SVPH-4b polypeptides (SEQ ID NOs:15-16) encodes a cytoplasmic domain. Due to alternative splicing the cytoplasmic domain of each polypeptide is different. The cytoplasmic domain of SVPH-4a is (Asn 714 to Lys 790), and the cytoplasmic domain of SVPH-4b is (Asn 714 to Lys 781).

The skilled artisan will recognize that the above-described boundaries of such regions of the polypeptides are approximate [illegible] are described above.

The polypeptides of the invention may be membrane bound or they may be [illegible]

the cells in which they are expressed. In general, soluble polypeptides may be identified (and distinguished from non-soluble membrane-bound counterparts) by separating intact cells which express the desired polypeptide from the culture medium, *e.g.*, by centrifugation, and assaying the medium (supernatant) for the presence of the desired polypeptide. The presence of polypeptide in the medium indicates that the polypeptide was secreted from the cells and thus is a soluble form of the protein.

In one embodiment, the soluble polypeptides and fragments thereof comprise all or part of the extracellular domain, but lack the transmembrane region that would cause retention of the polypeptide on a cell membrane. A soluble polypeptide may include the cytoplasmic domain, or a portion thereof, as long as the polypeptide is secreted from the cell in which it is produced.

In general, the use of soluble forms is advantageous for certain applications. Purification of the polypeptides from recombinant host cells is facilitated, since the soluble polypeptides are secreted from the cells. Further, soluble polypeptides are generally more suitable for intravenous administration.

The invention also provides polypeptides and fragments of the extracellular domain that retain a desired biological activity. Particular embodiments are directed to polypeptide fragments that retain the ability to bind the "binding partner" or the native cognates, substrates, or counter-structure. Such a fragment may be a soluble polypeptide, as described above. In another embodiment, the polypeptides and fragments advantageously include regions that are conserved in the SVPH family as described above.

Also provided herein are polypeptide fragments comprising at least 20, or at least 30, contiguous amino acids of the sequences of SEQ ID NOs:4-6 and 12-16. Fragments derived from the cytoplasmic domain find use in studies of signal transduction, and in regulating cellular processes associated with transduction of biological signals. Polypeptide fragments also may be employed as immunogens, in

Naturally occurring variants as well as derived variants of the polypeptides and

Variants may exhibit amino acid sequences that are at least 80% identical. Also contemplated are embodiments in which a polypeptide or fragment comprises an amino acid sequence that is at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, or at least 99.9% identical to the preferred polypeptide or fragment thereof. Percent identity may be determined by visual inspection and mathematical calculation. Alternatively, the percent identity of two protein sequences can be determined by comparing sequence information using the GAP computer program, based on the algorithm of Needleman and Wunsch, *J. Mol. Biol.*, 48:443 (1970) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program include: (1) a scoring matrix, blosum62, as described by Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA*, 89:10915 (1992); (2) a gap weight of 12; (3) a gap length weight of 4; and (4) no penalty for end gaps. Other programs used by one skilled in the art of sequence comparison may also be used.

The variants of the invention include, for example, those that result from alternate mRNA splicing events or from proteolytic cleavage. Alternate splicing of mRNA may, for example, yield a truncated but biologically active protein, such as a naturally occurring soluble form of the protein. Variations attributable to proteolysis include, for example, differences in the N- or C-termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the protein (generally from 1-5 terminal amino acids). Proteins in which differences in amino acid sequence are attributable to genetic polymorphism (allelic variation among individuals producing the protein) are also contemplated herein.

Additional variants within the scope of the invention include polypeptides that may be modified to create derivatives thereof by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives may be prepared by linking the

[illegible] are contemplated herein, as discussed in more detail below.

Other derivatives include covalent or aggregative conjugates of the polypeptides with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. Examples of fusion proteins are discussed below in connection with oligomers. Further, fusion proteins can comprise peptides added to facilitate purification and identification. Such peptides include, for example, poly-His or the antigenic identification peptides described in U.S. Patent No. 5,011,912 and in Hopp et al., *Bio/Technology* 6:1204 (1988). One such peptide is the FLAG® peptide, Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (SEQ ID NO:28), which is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. A murine hybridoma designated 4E11 produces a monoclonal antibody that binds the FLAG® peptide in the presence of certain divalent metal cations, as described in U.S. Patent 5,011,912, hereby incorporated by reference. The 4E11 hybridoma cell line has been deposited with the American Type Culture Collection under accession no. HB 9259. Monoclonal antibodies that bind the FLAG® peptide are available from Eastman Kodak Co., Scientific Imaging Systems Division, New Haven, Connecticut.

Among the variant polypeptides provided herein are variants of native polypeptides that retain the native biological activity or the substantial equivalent thereof. One example is a variant that binds with essentially the same binding affinity as does the native form. Binding affinity can be measured by conventional procedures, *e.g.*, as described in U.S. Patent No. 5,512,457 and as set forth below.

Variants include polypeptides that are substantially homologous to the native form, but which have an amino acid sequence different from that of the native form because of one or more deletions, insertions or substitutions. Particular embodiments include, but are not limited to, polypeptides that comprise from one to ten deletions, insertions or substitutions of amino acid residues, when compared to a native

[illegible] substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one

Glu and Asp, or Gln and Asn; or substitutions of one aromatic residue for another, such as Phe, Trp, or Tyr for one another. Other conservative substitutions, *e.g.*, involving substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Similarly, the DNAs of the invention include variants that differ from a native DNA sequence because of one or more deletions, insertions or substitutions, but that encode a biologically active polypeptide.

The invention further includes polypeptides of the invention with or without associated native-pattern glycosylation. Polypeptides expressed in yeast or mammalian expression systems (*e.g.*, COS-1 or COS-7 cells) can be similar to or significantly different from a native polypeptide in molecular weight and glycosylation pattern, depending upon the choice of expression system. Expression of polypeptides of the invention in bacterial expression systems, such as *E. coli*, provides non-glycosylated molecules. Further, a given preparation may include multiple differentially glycosylated species of the protein. Glycosyl groups can be removed through conventional methods, in particular those utilizing glycopeptidase. In general, glycosylated polypeptides of the invention can be incubated with a molar excess of glycopeptidase (Boehringer Mannheim).

Correspondingly, similar DNA constructs that encode various additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences are encompassed by the invention. For example, N-glycosylation sites in the polypeptide extracellular domain can be modified to preclude glycosylation, allowing expression of a reduced carbohydrate analog in mammalian and yeast expression systems. N-glycosylation sites in eukaryotic polypeptides are characterized by an amino acid triplet Asn-X-Y, wherein X is any amino acid except Pro and Y is Ser or Thr. Appropriate substitutions, additions, or deletions to the nucleotide sequence encoding these triplets will result in prevention of

sufficient to inactivate an N-glycosylation site. Alternatively, the Ser or Thr can be replaced with another amino acid, such as Ala. Known procedures for inactivating N-

glycosylation sites in proteins include those described in U.S. Patent 5,071,972 and EP 276,846, hereby incorporated by reference.

In another example of variants, sequences encoding Cys residues that are not essential for biological activity can be altered to cause the Cys residues to be deleted or replaced with other amino acids, preventing formation of incorrect intramolecular disulfide bridges upon folding or renaturation.

Other variants are prepared by modification of adjacent dibasic amino acid residues, to enhance expression in yeast systems in which KEX2 protease activity is present. EP 212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a protein. KEX2 protease processing sites are inactivated by deleting, adding or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. Lys-Lys pairings are considerably less susceptible to KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites.

Oligomers

Encompassed by the invention are oligomers or fusion proteins that contain SVPH polypeptides. When the polypeptide of the invention is a type I membrane protein, such as SVPH, the fusion partner is linked to the C terminus of the type I membrane protein. Such oligomers may be in the form of covalently-linked or non-covalently-linked multimers, including dimers, trimers, or higher oligomers. As noted above, preferred polypeptides are soluble and thus these oligomers may comprise soluble polypeptides. In one aspect of the invention, the oligomers maintain the binding ability of the polypeptide components and provide therefor, bivalent, trivalent, etc., binding sites.

One embodiment of the invention is directed to oligomers comprising multiple

peptides that have the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote

oligomerization of the polypeptides attached thereto, as described in more detail below.

Immunoglobulin-based Oligomers

As one alternative, an oligomer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, *e.g.*, by Ashkenazi et al., *PNAS USA*, 88:10535 (1991); Byrn et al., *Nature*, 344:677 (1990); and Hollenbaugh and Aruffo, "Construction of Immunoglobulin Fusion Proteins", *Current Protocols in Immunology*, Suppl. 4, pages 10.19.1 - 10.19.11 (1992).

One embodiment of the present invention is directed to a dimer comprising two fusion proteins created by fusing a polypeptide of the invention to an Fc polypeptide derived from an antibody. A gene fusion encoding the polypeptide/Fc fusion protein is inserted into an appropriate expression vector. Polypeptide/Fc fusion proteins are expressed in host cells transformed with the recombinant expression vector, and allowed to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield divalent molecules.

The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides made up of the Fc region of an antibody comprising any or all of the CH domains of the Fc region. Truncated forms of such polypeptides containing the hinge region that promotes dimerization are also included. Preferred polypeptides comprise an Fc polypeptide derived from a human IgG1 antibody.

One suitable Fc polypeptide, described in PCT application WO 93/10151 (hereby incorporated by reference), is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Patent 5,457,035 and in Baum et al., *EMBO J.* 13:3992-4001 (1994), incorporated herein by

from Leu to Val, amino acid [illegible] has been changed from Leu to Glu, and amino acid [illegible] has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc

The above-described fusion proteins comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

In other embodiments, the polypeptides of the invention may be substituted for the variable portion of an antibody heavy or light chain. If fusion proteins are made with both heavy and light chains of an antibody, it is possible to form an oligomer with as many as four SVPH extracellular regions.

Peptide-linker Based Oligomers

Alternatively, the oligomer is a fusion protein comprising multiple polypeptides, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233, which are hereby incorporated by reference. A DNA sequence encoding a desired peptide linker may be inserted between, and in the same reading frame as, the DNA sequences of the invention, using any suitable conventional technique. For example, a chemically synthesized oligonucleotide encoding the linker may be ligated between the sequences. In particular embodiments, a fusion protein comprises from two to four soluble SVPH polypeptides, separated by peptide linkers.

Leucine-Zippers

Another method for preparing the oligomers of the invention involves use of a leucine zipper. Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science*, 240:1759 (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize.

The zipper domain (also referred to herein as an oligomerizing, or oligomer-

found in the yeast transcription factor GCN4 and a heat-stable DNA-binding protein found in rat liver, C/EBP, (Landschulz et al., *Science* 243:1681 (1989)). Two nuclear

product of the murine proto-oncogene, c-myc (Landschulz et al., *Science* 240:1759 (1988)). The zipper domains of *fos* and *jun* preferentially form heterodimer (O'Shea et al., *Science* 245:646 (1989), Turner and Tjian, *Science*, 243:1689 (1989)). The zipper domain is necessary for biological activity (DNA binding) in these proteins.

The fusogenic proteins of several different viruses, including paramyxovirus, coronavirus, measles virus and many retroviruses, also possess zipper domains (Buckland and Wild, *Nature* 338:547 (1989); Britton, *Nature*, 353:394 (1991); Delwart and Mosialos, *AIDS Research and Human Retroviruses* 6:703 (1990)). The zipper domains in these fusogenic viral proteins are near the transmembrane region of the proteins; it has been suggested that the zipper domains could contribute to the oligomeric structure of the fusogenic proteins. Oligomerization of fusogenic viral proteins is involved in fusion pore formation (Spruce et al., *Proc. Natl. Acad. Sci. U.S.A.*, 88:3523 (1991)). Zipper domains have also been recently reported to play a role in oligomerization of heat-shock transcription factors (Rabindran et al., *Science* 259:230 (1993)).

Zipper domains fold as short, parallel coiled coils (O'Shea et al., *Science* 254:539 (1991)). The general architecture of the parallel coiled coil has been well characterized, with a "knobs-into-holes" packing as proposed by Crick, *Acta Crystallogr.*, 6:689 (1953). The dimer formed by a zipper domain is stabilized by the heptad repeat, designated $(abcdefg)_n$ according to the notation of McLachlan and Stewart, *J. Mol. Biol.*, 98:293 (1975), in which residues *a* and *d* are generally hydrophobic residues, with *d* being a leucine, which line up on the same face of a helix. Oppositely-charged residues commonly occur at positions *g* and *e*. Thus, in a parallel coiled coil formed from two helical zipper domains, the "knobs" formed by the hydrophobic side chains of the first helix are packed into the "holes" formed between the side chains of the second helix.

The residues at position *d* (often leucine) contribute large hydrophobic

reported the synthesis of a triple-stranded α-helical bundle in which the helices run up-up-down. Their studies confirmed that hydrophobic stabilization energy provides

studies also indicate that electrostatic interactions contribute to the stoichiometry and geometry of coiled coils. Further discussion of the structure of leucine zippers is found in Harbury et al., *Science*, 262:1401 (1993).

Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in PCT application WO 94/10308, as well as the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe et al., *FEBS Letters*, 344:191 (1994), hereby incorporated by reference. The use of a modified leucine zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow et al., *Semin. Immunol.*, 6:267-278 (1994). Recombinant fusion proteins comprising a soluble polypeptide fused to a leucine zipper peptide are expressed in suitable host cells, and the soluble oligomer that forms is recovered from the culture supernatant.

Certain leucine zipper moieties preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD) noted above, as described in Hoppe et al., *FEBS Letters*, 344:191 (1994) and in U.S. Patent 5,716,805, hereby incorporated by reference in their entirety. This lung SPD-derived leucine zipper peptide comprises the amino acid sequence Pro Asp Val Ala Ser Leu Arg Gln Gln Val Glu Ala Leu Gln Gly Gln Val Gln His Leu Gln Ala Ala Phe Ser Gln Tyr (SEQ ID NO:29).

Another example of a leucine zipper that promotes trimerization is a peptide comprising the amino acid sequence Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu Arg (SEQ ID NO:30), as described in U.S. Patent 5,716,805. In one alternative embodiment, an N-terminal Asp residue is added; in another, the peptide lacks the N-terminal Arg residue.

Fragments of the foregoing zipper peptides that retain the property of promoting oligomerization may be employed as well. Examples of such fragments

may be derived from naturally occurring leucine zipper peptides, e.g., via conservative substitution(s) in the native amino acid sequence, wherein the peptide's ability to

Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric SVPH. Alternatively, synthetic peptides that promote oligomerization may be employed. In particular embodiments, leucine residues in a leucine zipper moiety are replaced by isoleucine residues. Such peptides comprising isoleucine may be referred to as isoleucine zippers, but are encompassed by the term "leucine zippers" as employed herein.

PRODUCTION OF POLYPEPTIDES AND FRAGMENTS THEREOF

Expression, isolation and purification of the polypeptides and fragments of the invention may be accomplished by any suitable technique, including but not limited to the following:

Expression Systems

The present invention also provides recombinant cloning and expression vectors containing DNA, as well as host cell containing the recombinant vectors. Expression vectors comprising DNA may be used to prepare the polypeptides or fragments of the invention encoded by the DNA. A method for producing polypeptides comprises culturing host cells transformed with a recombinant expression vector encoding the polypeptide, under conditions that promote expression of the polypeptide, then recovering the expressed polypeptides from the culture. The skilled artisan will recognize that the procedure for purifying the expressed polypeptides will vary according to such factors as the type of host cells employed, and whether the polypeptide is membrane-bound or a soluble form that is secreted from the host cell.

Any suitable expression system may be employed. The vectors include a DNA encoding a polypeptide or fragment of the invention, operably linked to suitable transcriptional or translational regulatory nucleotide sequences, such as those derived

ribosomal binding site, and appropriate sequences which control transcription and translation initiation and termination. Nucleotide sequences are operably linked when

nucleotide sequence is operably linked to a DNA sequence if the promoter nucleotide sequence controls the transcription of the DNA sequence. An origin of replication that confers the ability to replicate in the desired host cells, and a selection gene by which transformants are identified, are generally incorporated into the expression vector.

In addition, a sequence encoding an appropriate signal peptide (native or heterologous) can be incorporated into expression vectors. A DNA sequence for a signal peptide (secretory leader) may be fused in frame to the nucleic acid sequence of the invention so that the DNA is initially transcribed, and the mRNA translated, into a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cells promotes extracellular secretion of the polypeptide. The signal peptide is cleaved from the polypeptide upon secretion of polypeptide from the cell.

The skilled artisan will also recognize that the position(s) at which the signal peptide is cleaved may differ from that predicted by computer program, and may vary according to such factors as the type of host cells employed in expressing a recombinant polypeptide. A protein preparation may include a mixture of protein molecules having different N-terminal amino acids, resulting from cleavage of the signal peptide at more than one site.

Suitable host cells for expression of polypeptides include prokaryotes, yeast or higher eukaryotic cells. Mammalian or insect cells are generally preferred for use as host cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described, for example, in Pouwels et al. *Cloning Vectors: A Laboratory Manual*, Elsevier, New York, (1985). Cell-free translation systems could also be employed to produce polypeptides using RNAs derived from DNA constructs disclosed herein.

Prokaryotic Systems

subtilis, *Salmonella typhimurium*, and various other species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*. In a prokaryotic host cell, such as

expression of the recombinant polypeptide in the prokaryotic host cell. The N-terminal Met may be cleaved from the expressed recombinant polypeptide.

Expression vectors for use in prokaryotic host cells generally comprise one or more phenotypic selectable marker genes. A phenotypic selectable marker gene is, for example, a gene encoding a protein that confers antibiotic resistance or that supplies an autotrophic requirement. Examples of useful expression vectors for prokaryotic host cells include those derived from commercially available plasmids such as the cloning vector pBR322 (ATCC 37017). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells. An appropriate promoter and a DNA sequence are inserted into the pBR322 vector. Other commercially available vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA).

Promoter sequences commonly used for recombinant prokaryotic host cell expression vectors include β-lactamase (penicillinase), lactose promoter system (Chang et al., *Nature, 275*:615 (1978); and Goeddel et al., *Nature, 281*:544 (1979)), tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res., 8*:4057 (1980)); and EP-A-36776 and tac promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412 (1982)). A particularly useful prokaryotic host cell expression system employs a phage λP_L promoter and a cI857ts thermolabile repressor sequence. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λP_L promoter include plasmid pHUB2 (resident in *E. coli* strain JMB9, ATCC 37092) and pPLc28 (resident in *E. coli* RR1, ATCC 53082).

Yeast Systems

Alternatively, the polypeptides may be expressed in yeast host cells, preferably

origin of replication sequence from a 2μ yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for

for yeast vectors include, among others, promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem. 255*:2073 (1980)) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg., 7*:149 (1968); and Holland et al., *Biochem., 17*:4900 (1978)), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phospho-glucose isomerase, and glucokinase. Other suitable vectors and promoters for use in yeast expression are further described in Hitzeman, EPA-73,657. Another alternative is the glucose-repressible ADH2 promoter described by Russell et al., *J. Biol. Chem., 258*:2674 (1982) and Beier et al., *Nature, 300*:724 (1982). Shuttle vectors replicable in both yeast and *E. coli* may be constructed by inserting DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) into the above-described yeast vectors.

The yeast α-factor leader sequence may be employed to direct secretion of the polypeptide. The α-factor leader sequence is often inserted between the promoter sequence and the structural gene sequence. *See*, Kurjan et al., *Cell, 30*:933 (1982) and Bitter et al., *Proc. Natl. Acad. Sci. USA, 81*:5330 (1984). Other leader sequences suitable for facilitating secretion of recombinant polypeptides from yeast hosts are known to those of skill in the art. A leader sequence may be modified near its 3' end to contain one or more restriction sites. This will facilitate fusion of the leader sequence to the structural gene.

Yeast transformation protocols are known to those of skill in the art. One such protocol is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA, 75*:1929 (1978). The Hinnen et al. protocol selects for Trp$^+$ transformants in a selective medium, wherein the selective medium consists of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 mg/ml adenine and 20 mg/ml uracil.

Yeast host cells transformed by vectors containing an ADH2 promoter

supplemented with 80 mg/ml adenine and 80 mg/ml uracil. Derepression of the ADH2 promoter occurs when glucose is exhausted from the medium.

Mammalian or Insect Systems

Mammalian or insect host cell culture systems also may be employed to express recombinant polypeptides. Bacculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Established cell lines of mammalian origin also may be employed. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., *Cell 23*:175 (1981)), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, and BHK (ATCC CRL 10) cell lines, and the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) as described by McMahan et al., *EMBO J.* 10: 2821 (1991).

Established methods for introducing DNA into mammalian cells have been described (Kaufman, R.J., *Large Scale Mammalian Cell Culture*, pp. 15-69 (1990)). Additional protocols using commercially available reagents, such as Lipofectamine lipid reagent (Gibco/BRL) or Lipofectamine-Plus lipid reagent, can be used to transfect cells (Felgner et al., *Proc. Natl. Acad. Sci. USA 84*:7413-7417 (1987)). In addition, electroporation can be used to transfect mammalian cells using conventional procedures, such as those in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed. Vol. 1-3, Cold Spring Harbor Laboratory Press (1989). Selection of stable transformants can be performed using methods known in the art, such as, for example, resistance to cytotoxic drugs. Kaufman et al., *Meth. in Enzymology 185*:487-511 (1990), describes several selection schemes, such as dihydrofolate reductase (DHFR) resistance. A suitable host strain for DHFR selection can be CHO strain DX-B11, which is deficient in DHFR (Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA 77*:4216-4220 (1980)). A plasmid expressing the DHFR cDNA can be introduced into strain DX-B11, and only cells that contain the plasmid can grow in the appropriate selective media. Other examples of selectable markers that can be

selected on the basis of resistance to these compounds.

Transcriptional and translational control sequences for mammalian host cell

sequences and enhancer sequences are derived from polyoma virus, adenovirus 2, simian virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites can be used to provide other genetic elements for expression of a structural gene sequence in a mammalian host cell. Viral early and late promoters are particularly useful because both are easily obtained from a viral genome as a fragment, which can also contain a viral origin of replication (Fiers et al., *Nature 273*:113 (1978); Kaufman et al., *Meth. in Enzymology 185*:487-511 (1990)). Smaller or larger SV40 fragments can also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* I site located in the SV40 viral origin of replication site is included.

Additional control sequences shown to improve expression of heterologous genes from mammalian expression vectors include such elements as the expression augmenting sequence element (EASE) derived from CHO cells (Morris et al., *Animal Cell Technology*, pp. 529-534 (1997)) and PCT Application WO 97/25420 and the tripartite leader (TPL) and VA gene RNAs from Adenovirus 2 (Gingeras et al., *J. Biol. Chem. 257*:13475-13491 (1982)). The internal ribosome entry site (IRES) sequences of viral origin allows dicistronic mRNAs to be translated efficiently (Oh and Sarnow, *Current Opinion in Genetics and Development 3*:295-300 (1993); Ramesh et al., *Nucleic Acids Research 24*:2697-2700 (1996)). Expression of a heterologous cDNA as part of a dicistronic mRNA followed by the gene for a selectable marker (e.g. DHFR) has been shown to improve transfectability of the host and expression of the heterologous cDNA (Kaufman et al., *Meth. in Enzymology 185*:487-511 (1990)). Exemplary expression vectors that employ dicistronic mRNAs are pTR-DC/GFP described by Mosser et al., *Biotechniques* 22:150-161 (1997), and p2A5I described by Morris et al., *Animal Cell Technology*, pp. 529-534 (1997).

A useful high expression vector, pCAVNOT, has been described by Mosley et

A useful system for stable high level expression of mammalian cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by

PMLSV N1/N4, described by Cosman et al., *Nature 312*:768 (1984), has been deposited as ATCC 39890. Additional useful mammalian expression vectors are described in EP-A-0367566, and in WO 91/18982, incorporated by reference herein. In yet another alternative, the vectors can be derived from retroviruses.

Additional useful expression vectors, pFLAG® and pDC311, can also be used. FLAG® technology is centered on the fusion of a low molecular weight (1kD), hydrophilic, FLAG® marker peptide to the N-terminus of a recombinant protein expressed by pFLAG® expression vectors. pDC311 is another specialized vector used for expressing proteins in CHO cells. pDC311 is characterized by a bicistronic sequence containing the gene of interest and a dihydrofolate reductase (DHFR) gene with an internal ribosome binding site for DHFR translation, an expression augmenting sequence element (EASE), the human CMV promoter, a tripartite leader sequence, and a polyadenylation site.

Regarding signal peptides that may be employed, the native signal peptide may be replaced by a heterologous signal peptide or leader sequence, if desired. The choice of signal peptide or leader may depend on factors such as the type of host cells in which the recombinant polypeptide is to be produced. To illustrate, examples of heterologous signal peptides that are functional in mammalian host cells include the signal sequence for interleukin-7 (IL-7) described in United States Patent 4,965,195; the signal sequence for interleukin-2 receptor described in Cosman et al., *Nature*, 312:768 (1984); the interleukin-4 receptor signal peptide described in EP 367,566; the type I interleukin-1 receptor signal peptide described in U.S. Patent 4,968,607; and the type II interleukin-1 receptor signal peptide described in EP 460,846.

Purification

The invention also includes methods of isolating and purifying the polypeptides and fragments thereof.

Isolation and Purification

The "isolated" polypeptides or fragments thereof encompassed by this invention are polypeptides or fragments that are not in an environment identical to an environment in which it or they can be found in nature. The "purified" polypeptides or fragments thereof encompassed by this invention are essentially free of association with other proteins or polypeptides, for example, as a purification product of recombinant expression systems such as those described above or as a purified product from a non-recombinant source such as naturally occurring cells and/or tissues.

In one preferred embodiment, the purification of recombinant polypeptides or fragments can be accomplished using fusions of polypeptides or fragments of the invention to another polypeptide to aid in the purification of polypeptides or fragments of the invention. Such fusion partners can include the poly-His or other antigenic identification peptides described above as well as the Fc moieties described previously.

With respect to any type of host cell, as is known to the skilled artisan, procedures for purifying a recombinant polypeptide or fragment will vary according to such factors as the type of host cells employed and whether or not the recombinant polypeptide or fragment is secreted into the culture medium.

In general, the recombinant polypeptide or fragment can be isolated from the host cells if not secreted, or from the medium or supernatant if soluble and secreted, followed by one or more concentration, salting-out, ion exchange, hydrophobic interaction, affinity purification or size exclusion chromatography steps. As to specific ways to accomplish these steps, the culture medium first can be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium.

[illegible] acrylamide, agarose, dextran, cellulose or other type commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation [illegible]

carboxymethyl groups. In addition, a chromatofocusing step can be employed. Alternatively, a hydrophobic interaction chromatography step can be employed. Suitable matrices can be phenyl or octyl moieties bound to resins. In addition, affinity chromatography with a matrix which selectively binds the recombinant protein can be employed. Examples of such resins employed are lectin columns, dye columns, and metal-chelating columns. Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, (e.g., silica gel or polymer resin having pendant methyl, octyl, octyldecyl or other aliphatic groups) can be employed to further purify the polypeptides. Some or all of the foregoing purification steps, in various combinations, are well known and can be employed to provide an isolated and purified recombinant protein.

It is also possible to utilize an affinity column comprising a polypeptide-binding protein of the invention, such as a monoclonal antibody generated against polypeptides of the invention, to affinity-purify expressed polypeptides. These polypeptides can be removed from an affinity column using conventional techniques, e.g., in a high salt elution buffer and then dialyzed into a lower salt buffer for use or by changing pH or other components depending on the affinity matrix utilized, or be competitively removed using the naturally occurring substrate of the affinity moiety, such as a polypeptide derived from the invention.

In this aspect of the invention, polypeptide-binding proteins, such as the anti-polypeptide antibodies of the invention or other proteins that may interact with the polypeptide of the invention, can be bound to a solid phase support such as a column chromatography matrix or a similar substrate suitable for identifying, separating, or purifying cells that express polypeptides of the invention on their surface. Adherence of polypeptide-binding proteins of the invention to a solid phase contacting surface can be accomplished by any means, for example, magnetic microspheres can be coated with these polypeptide-binding proteins and held in the incubation vessel through a magnetic field. Suspensions of cell mixtures are contacted with the solid

unbound cells then are washed away. This affinity-binding method is useful for

Methods of releasing positively selected cells from the solid phase are known in the art and encompass, for example, the use of enzymes. Such enzymes are preferably non-toxic and non-injurious to the cells and are preferably directed to cleaving the cell-surface binding partner.

Alternatively, mixtures of cells suspected of containing polypeptide-expressing cells of the invention first can be incubated with a biotinylated polypeptide-binding protein of the invention. Incubation periods are typically at least one hour in duration to ensure sufficient binding to polypeptides of the invention. The resulting mixture then is passed through a column packed with avidin-coated beads, whereby the high affinity of biotin for avidin provides the binding of the polypeptide-binding cells to the beads. Use of avidin-coated beads is known in the art. *See*, Berenson, et al., *J. Cell. Biochem.*, 10D:239 (1986). Wash of unbound material and the release of the bound cells is performed using conventional methods.

The desired degree of purity depends on the intended use of the protein. A relatively high degree of purity is desired when the polypeptide is to be administered *in vivo*, for example. In such a case, the polypeptides are purified such that no protein bands corresponding to other proteins are detectable upon analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). It will be recognized by one skilled in the pertinent field that multiple bands corresponding to the polypeptide may be visualized by SDS-PAGE, due to differential glycosylation, differential post-translational processing, and the like. Most preferably, the polypeptide of the invention is purified to substantial homogeneity, as indicated by a single protein band upon analysis by SDS-PAGE. The protein band may be visualized by silver staining, Coomassie blue staining, or (if the protein is radiolabeled) by autoradiography.

Assays

The purified polypeptides of the invention (including proteins, polypeptides,

fragments, the polypeptide may be labeled with a detectable reagent (e.g., a radionuclide, chromophore, enzyme that catalyzes a colorimetric or fluorometric

binding partner. The cells then are washed to remove unbound labeled polypeptide, and the presence of cell-bound label is determined by a suitable technique, chosen according to the nature of the label.

One example of a binding assay procedure is as follows. A recombinant expression vector containing binding partner cDNA is constructed using methods well known in the art. The binding partner comprises an N-terminal cytoplasmic domain, a transmembrane region, and a C-terminal extracellular domain. CV1-EBNA-1 cells in 10 cm^2 dishes are transfected with the recombinant expression vector. CV-1/EBNA-1 cells (ATCC CRL 10478) constitutively express EBV nuclear antigen-1 driven from the CMV immediate-early enhancer/promoter. CV1-EBNA-1 was derived from the African Green Monkey kidney cell line CV-1 (ATCC CCL 70), as described by McMahan et al., *EMBO J.*, 10:2821 (1991).

The transfected cells are cultured for 24 hours, and the cells in each dish then are split into a 24-well plate. After culturing an additional 48 hours, the transfected cells (about 4 x 10^4 cells/well) are washed with BM-NFDM, which is binding medium (RPMI 1640 containing 25 mg/ml bovine serum albumin, 2 mg/ml sodium azide, 20 mM Hepes pH 7.2) to which 50 mg/ml nonfat dry milk has been added. The cells then are incubated for 1 hour at 37°C with various concentrations of, for example, a soluble polypeptide/Fc fusion protein made as set forth above. Cells then are washed and incubated with a constant saturating concentration of a ^{125}I-mouse anti-human IgG in binding medium, with gentle agitation for 1 hour at 37°C. After extensive washing, cells are released *via* trypsinization.

The mouse anti-human IgG employed above is directed against the Fc region of human IgG and can be obtained from Jackson Immunoresearch Laboratories, Inc., West Grove, PA. The antibody is radioiodinated using the standard chloramine-T method. The antibody will bind to the Fc portion of any polypeptide/Fc protein that has bound to the cells. In all assays, non-specific binding of ^{125}I-antibody is assayed

[illegible] antibody is quantified on a Packard Minigamma counter.

Affinity calculations (Scatchard, *Ann. N.Y. Acad. Sci.*, 51:660 (1949)) are generated

Another type of suitable binding assay is a competitive binding assay. To illustrate, biological activity of a variant may be determined by assaying for the variant's ability to compete with the native protein for binding to the binding partner.

Competitive binding assays can be performed by conventional methodology. Reagents that may be employed in competitive binding assays include radiolabeled SVPH and intact cells expressing the binding partner (endogenous or recombinant) on the cell surface. For example, a radiolabeled soluble SVPH fragment can be used to compete with a soluble SVPH variant for binding to cells expressing the binding partner on the surface. Instead of intact cells, one could substitute a soluble binding partner/Fc fusion protein bound to a solid phase through the interaction of Protein A or Protein G (on the solid phase) with the Fc moiety. Chromatography columns that contain Protein A and Protein G include those available from Pharmacia Biotech, Inc., Piscataway, NJ.

Another type of competitive binding assay utilizes radiolabeled soluble binding partner, such as a soluble binding partner/Fc fusion protein, and intact cells expressing SVPH. Qualitative results can be obtained by competitive autoradiographic plate binding assays, while (Scatchard plots Scatchard, *Ann. N.Y. Acad. Sci.* 51:660 (1949)) may be utilized to generate quantitative results.

USE OF SVPH NUCLEIC ACID OR OLIGONUCLEOTIDES

In addition to being used to express polypeptides as described above, the nucleic acids of the invention, including DNA, RNA, mRNA, and oligonucleotides thereof can be used:

- as probes to identify nucleic acid encoding proteins having proteinase activity;
- to identify human chromosome number 1 or 4;
- to map genes on human chromosome number 1 or 4;

- as single-stranded sense or antisense oligonucleotides, to inhibit expression of polypeptides encoded by the SVPH-1, SVPH-3, or SVPH-4 gene;
- to detect defective genes in an individual; and
- for gene therapy.

Probes

The nucleotides of the invention can be used as probes to identify nucleic acid encoding proteins having similar activity or structure. Such uses include the use of fragments. Such fragments may comprise any length of contiguous nucleotides. In one embodiment, the fragment comprises at least about 17 contiguous nucleotides of a DNA sequence. In other embodiments, a DNA fragment comprises at least 30, or at least 60, contiguous nucleotides of a DNA sequence.

Because homologs of SEQ ID NOs:1-3 and 7-11, from other mammalian species are contemplated herein, probes based on the human DNA sequence of SEQ ID NOs:1-3 and 7-11 may be used to screen cDNA libraries derived from other mammalian species, using conventional cross-species hybridization techniques.

Using knowledge of the genetic code in combination with the amino acid sequences set forth above, sets of degenerate oligonucleotides can be prepared. Such oligonucleotides are useful as primers, e.g., in polymerase chain reactions (PCR), whereby DNA fragments are isolated and amplified.

Chromosome Mapping

All or a portion of the nucleic acids of SEQ ID NOs:1-3 and 7-11, including oligonucleotides, can be used by those skilled in the art using well-known techniques to identify human chromosomes and the specific locus thereof, that contains the DNA of SVPH family members. For example, all or a portion of SEQ ID NO:3, SEQ ID

are not limited to, using the sequence or portions, including oligonucleotides, as a

resolution), in situ hybridization to chromosome spreads (moderate resolution), and Southern blot hybridization to hybrid cell lines containing individual human chromosomes (low resolution).

For example, chromosomes can be mapped by radiation hybrid mapping. First, PCR is performed using the Whitehead Institute/MIT Center for Genome Research Genebridge4 panel of 93 radiation hybrids (http://www-genome.wi.mit.edu/ftp/distribution/human_STS_releases/july97/rhmap/genebridge4.html). Primers are used which lie within a putative exon of the gene of interest and which amplify a product from human genomic DNA, but do not amplify hamster genomic DNA. The results of the PCRs are converted into a data vector that is submitted to the Whitehead/MIT Radiation Mapping site on the internet (http://www-seq.wi.mit.edu). The data is scored and the chromosomal assignment and placement relative to known Sequence Tag Site (STS) markers on the radiation hybrid map is provided. The following web site provides additional information about radiation hybrid mapping: http://www-genome.wi.mit.edu/ftp/distribution/human_STS_releases/july97/07-97.INTRO.html).

Identifying Associated Diseases

As set forth below, sequences encoding SVPH-4a and SVPH-4b have been mapped by radiation hybrid mapping to the 1p11-13 region of chromosome 1. That region is associated with specific diseases which include but are not limited to fetal hydantoin syndrome, diphenylhydantoin toxicity, and pheochromocytoma. Thus, the nucleic acid of SEQ ID Nos:3, 10 and 11, or a fragment thereof, can be used by one skilled in the art using well-known techniques to analyze abnormalities associated with SVPH-4 genes. In addition, sequences encoding SVPH-1a, SVPH-1b, and SVPH-1c have been mapped by radiation hybrid mapping to the 4q34 region of

nucleic acid of SEQ ID No:2, or a fragment thereof, to analyze abnormalities

this marker is rearranged or deleted. In addition, nucleic acid of SEQ ID NOs:1-3 and 7-11 or a fragment thereof can be used as a positional marker to map other genes of unknown location.

The DNA may be used in developing treatments for any disorder mediated (directly or indirectly) by defective, or insufficient amounts of, the genes corresponding to the nucleic acids of the invention. Disclosure herein of native nucleotide sequences permits the detection of defective genes, and the replacement thereof with normal genes. Defective genes may be detected in *in vitro* diagnostic assays, and by comparison of a native nucleotide sequence disclosed herein with that of a gene derived from a person suspected of harboring a defect in this gene.

Sense-Antisense

Other useful fragments of the nucleic acids include antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target mRNA (sense) or DNA (antisense) sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of SEQ ID NOs:1-3 or 7-11. Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to about 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen, *Cancer Res.*, 48:2659 (1988) and van der Krol et al., *BioTechniques*, 6:958 (1988).

Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block or inhibit protein expression by one of several means, including enhanced degradation of the mRNA by RNAseH, inhibition of splicing, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of proteins. Antisense or sense oligonucleotides further comprise oligonucleotides having

in vivo (i.e., capable of resisting enzymatic degradation) but retain sequence

Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10448, and other moieties that increases affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, lipofection, $CaPO_4$-mediated DNA transfection, electroporation, or by using gene transfer vectors such as Epstein-Barr virus.

Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell.

Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

USE OF SVPH POLYPEPTIDES AND FRAGMENTED POLYPEPTIDES

Uses include, but are not limited to, the following:

- Purifying proteins and measuring activity thereof

- Molecular weight and isoelectric focusing markers

- Controls for peptide fragmentation

- Identification of unknown proteins
- Preparation of Antibodies

Purification Reagents

Each of the polypeptides of the invention finds use as a protein purification reagent. For example, the polypeptides may be used to purify binding partner proteins. In particular embodiments, a polypeptide (in any form described herein that is capable of binding the binding partner) is attached to a solid support by conventional procedures. As one example, affinity chromatography columns containing functional groups that will react with functional groups on amino acid side chains of proteins are available (Pharmacia Biotech, Inc., Piscataway, NJ). In an alternative, a polypeptide/Fc protein (as discussed above) is attached to Protein A- or Protein G-containing chromatography columns through interaction with the Fc moiety.

The polypeptide also finds use in purifying or identifying cells that express the binding partner on the cell surface. Polypeptides are bound to a solid phase such as a column chromatography matrix or a similar suitable substrate. For example, magnetic microspheres can be coated with the polypeptides and held in an incubation vessel through a magnetic field. Suspensions of cell mixtures containing the binding partner expressing cells are contacted with the solid phase having the polypeptides thereon. Cells expressing the binding partner on the cell surface bind to the fixed polypeptides, and unbound cells then are washed away.

Alternatively, the polypeptides can be conjugated to a detectable moiety, then incubated with cells to be tested for binding partner expression. After incubation, unbound labeled matter is removed and the presence or absence of the detectable moiety on the cells is determined.

In a further alternative, mixtures of cells suspected of containing cells [illegible] with biotinylated polypeptide [illegible] coated beads, whereby the high affinity of biotin for avidin provides binding of the [illegible]

Berenson, et al., *J. Cell. Biochem.*, 10D:239 (1986). Washing to remove unbound material, and the release of the bound cells, are performed using conventional methods.

Measuring Activity

Polypeptides also find use in measuring the biological activity of the binding partner protein in terms of their binding affinity. The polypeptides thus may be employed by those conducting "quality assurance" studies, e.g., to monitor shelf life and stability of protein under different conditions. For example, the polypeptides may be employed in a binding affinity study to measure the biological activity of a binding partner protein that has been stored at different temperatures, or produced in different cell types. The proteins also may be used to determine whether biological activity is retained after modification of a binding partner protein (e.g., chemical modification, truncation, mutation, etc.). The binding affinity of the modified binding partner protein is compared to that of an unmodified binding partner protein to detect any adverse impact of the modifications on biological activity of the binding partner. The biological activity of a binding partner protein thus can be ascertained before it is used in a research study, for example.

Delivery Agents

The polypeptides also find use as carriers for delivering agents attached thereto to cells bearing the binding partner (or to other cell types found to express the binding partner on the cell surface) in *in vitro* or *in vivo* procedures.

Detectable (diagnostic) and therapeutic agents that may be attached to a polypeptide include, but are not limited to, toxins, other cytotoxic agents, drugs, radionuclides, chromophores, enzymes that catalyze a colorimetric or fluorometric reaction, and the like, with the particular agent being chosen according to the intended

Radionuclides suitable for diagnostic use include, but are not limited to,

^{99m}Tc, ^{111}In, and ^{76}Br. Examples of radionuclides suitable for therapeutic use are ^{131}I, ^{211}At, ^{77}Br, ^{186}Re, ^{188}Re, ^{212}Pb, ^{212}Bi, ^{109}Pd, ^{64}Cu, and ^{67}Cu.

Such agents may be attached to the polypeptide by any suitable conventional procedure. The polypeptide comprises functional groups on amino acid side chains that can be reacted with functional groups on a desired agent to form covalent bonds, for example. Alternatively, the protein or agent may be derivatized to generate or attach a desired reactive functional group. The derivatization may involve attachment of one of the bifunctional coupling reagents available for attaching various molecules to proteins (Pierce Chemical Company, Rockford, Illinois). A number of techniques for radiolabeling proteins are known. Radionuclide metals may be attached to polypeptides by using a suitable bifunctional chelating agent, for example.

Conjugates comprising polypeptides and a suitable diagnostic or therapeutic agent (preferably covalently linked) are thus prepared. The conjugates are administered or otherwise employed in an amount appropriate for the particular application.

Therapeutic Agents

Polypeptides of the invention may be used in developing treatments for any disorder mediated (directly or indirectly) by defective, or insufficient amounts of the polypeptides. These polypeptides may be administered to a mammal afflicted with such a disorder.

The polypeptides may also be employed in inhibiting a biological activity of the binding partner, in *in vitro* or *in vivo* procedures. For example, a purified polypeptide may be used to inhibit binding of the binding partner to an endogenous cell surface binding partner. Biological effects that result from the binding of SVPH to endogenous binding partner thus are inhibited.

In addition, an SVPH binding partner may be administered to a mammal to [illegible] binding partner mediated disorder. Such binding partner mediated disorder [illegible]

Compositions of the present invention may contain a polypeptide in any form [illegible]

biologically active fragments. In particular embodiments, the composition comprises a soluble polypeptide or an oligomer comprising soluble SVPH polypeptides or SVPH binding partner polypeptides.

Compositions comprising an effective amount of a polypeptide of the present invention, in combination with other components such as a physiologically acceptable diluent, carrier, or excipient, are provided herein. The polypeptides can be formulated according to known methods used to prepare pharmaceutically useful compositions. They can be combined in admixture, either as the sole active material or with other known active materials suitable for a given indication, with pharmaceutically acceptable diluents (e.g., saline, Tris-HCl, acetate, and phosphate buffered solutions), preservatives (e.g., thimerosal, benzyl alcohol, parabens), emulsifiers, solubilizers, adjuvants and/or carriers. Suitable formulations for pharmaceutical compositions include those described in *Remington's Pharmaceutical Sciences*, 16th ed. 1980, Mack Publishing Company, Easton, PA.

In addition, such compositions can be complexed with polyethylene glycol (PEG), metal ions, or incorporated into polymeric compounds such as polyacetic acid, polyglycolic acid, hydrogels, dextran, etc., or incorporated into liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts or spheroblasts. Such compositions will influence the physical state, solubility, stability, rate of *in vivo* release, and rate of *in vivo* clearance, and are thus chosen according to the intended application.

The compositions of the invention can be administered in any suitable manner, e.g., topically, parenterally, or by inhalation. The term "parenteral" includes injection, e.g., by subcutaneous, intravenous, or intramuscular routes, also including localized administration, e.g., at a site of disease or injury. Sustained release from implants is also contemplated. One skilled in the pertinent art will recognize that suitable dosages will vary, depending upon such factors as the nature of the disorder to be treated, the

Compositions comprising nucleic acids in physiologically acceptable formulations are also contemplated. DNA may be formulated for injection, for example.

Research Agents

Another use of the polypeptide of the present invention is as a research tool for studying the biological effects that result from inhibiting binding partner/SVPH interactions on different cell types. Polypeptides also may be employed in *in vitro* assays for detecting the binding partner or SVPH or the interactions thereof.

Molecular Weight, Isoelectric Point Markers

The polypeptides of the present invention can be subjected to fragmentation into smaller peptides by chemical and enzymatic means, and the peptide fragments so produced can be used in the analysis of other proteins or polypeptides. For example, such peptide fragments can be used as peptide molecular weight markers, peptide isoelectric point markers, or in the analysis of the degree of peptide fragmentation. Thus, the invention also includes these polypeptides and peptide fragments, as well as kits to aid in the determination of the apparent molecular weight and isoelectric point of an unknown protein and kits to assess the degree of fragmentation of an unknown protein.

Although all methods of fragmentation are encompassed by the invention, chemical fragmentation is a preferred embodiment, and includes the use of cyanogen bromide to cleave under neutral or acidic conditions such that specific cleavage occurs at methionine residues (E. Gross, *Methods in Enz.*, 11:238-255 (1967)). This can further include additional steps, such as a carboxymethylation step to convert cysteine residues to an unreactive species. Table 1 summarizes the fragmentation pattern of SEQ ID NOs:12-16 following chemical cleavage with cyanogen bromide.

Endoproteinase Asp-N, or Endoproteinase Lys-C under conventional conditions to

cleave specifically on the carboxyl side of the asparagine residues present within the polypeptides of the invention. Arginylendo-peptidase can cleave specifically on the carboxyl side of the arginine residues present within these polypeptides. *Achromobacter* protease I can cleave specifically on the carboxyl side of the lysine residues present within the polypeptides (Sakiyama and Nakat, U.S. Patent No. 5,248,599; T. Masaki et al., *Biochim. Biophys. Acta, 660*:44-50 (1981); T. Masaki et al., *Biochim. Biophys. Acta, 660*:51-55 (1981)). Trypsin can cleave specifically on the carboxyl side of the arginine and lysine residues present within polypeptides of the invention. Enzymatic fragmentation may also occur with a protease that cleaves at multiple amino acid residues. For example, *Staphlococcus aureus* V8 protease can cleave specifically on the carboxyl side of the aspartic and glutamic acid residues present within polypeptides (D. W. Cleveland, *J. Biol. Chem., 3*:1102-1106 (1977)). Endoproteinase Asp-N can cleave specifically on the amino side of the asparagine residues present within polypeptides. Endoproteinase Lys-C can cleave specifically on the carboxyl side of the lysine residues present within polypeptides of the invention. Other enzymatic and chemical treatments can likewise be used to specifically fragment these polypeptides into a unique set of specific peptides.

Of course, the peptides and fragments of the polypeptides of the invention can also be produced by conventional recombinant processes and synthetic processes well known in the art. With regard to recombinant processes, the polypeptides and peptide fragments encompassed by invention can have variable molecular weights, depending upon the host cell in which they are expressed. Glycosylation of polypeptides and peptide fragments of the invention in various cell types can result in variations of the molecular weight of these pieces, depending upon the extent of modification. The size of these pieces can be most heterogeneous with fragments of polypeptide derived from the extracellular portion of the polypeptide. Consistent polypeptides and peptide fragments can be obtained by using polypeptides derived entirely from the

additional peptide sequences to both the amino and carboxyl terminal ends of

and carboxyl terminal ends of polypeptides of the invention can be used to enhance expression of these polypeptides or aid in the purification of the protein. In addition, fusions of additional peptide sequences at the amino and carboxyl terminal ends of polypeptides of the invention will alter some, but usually not all, of the fragmented peptides of the polypeptides generated by enzymatic or chemical treatment. Of course, mutations can be introduced into polypeptides of the invention using routine and known techniques of molecular biology. For example, a mutation can be designed so as to eliminate a site of proteolytic cleavage by a specific enzyme or a site of cleavage by a specific chemically induced fragmentation procedure. The elimination of the site will alter the peptide fingerprint of polypeptides of the invention upon fragmentation with the specific enzyme or chemical procedure.

When the invention relates to the use of fragmented peptide molecular weight markers, those markers are preferably at least 10 amino acids in size. More preferably, these fragmented peptide molecular weight markers are between 10 and 100 amino acids in size. Even more preferable are fragmented peptide molecular weight markers between 10 and 50 amino acids in size and especially between 10 and 35 amino acids in size. Most preferable are fragmented peptide molecular weight markers between 10 and 20 amino acids in size.

Because the unique amino acid sequence of each fragment specifies a molecular weight, these fragments can thereafter serve as molecular weight markers using such analysis techniques to assist in the determination of the molecular weight of an unknown protein, polypeptides or fragments thereof. The molecular weight markers of the invention serve particularly well as molecular weight markers for the estimation of the apparent molecular weight of proteins that have similar apparent molecular weights and, consequently, allow increased accuracy in the determination of apparent molecular weight of proteins.

Among the methods for determining molecular weight are sedimentation, gel

a gel containing sodium dodecyl sulfate and a concentration of acrylamide between 6-

conditions allows for increased accuracy. It is understood, of course, that many different techniques can be used for the determination of the molecular weight of an unknown protein using polypeptides of the invention, and that this embodiment in no way limits the scope of the invention.

In addition, each unglycosylated polypeptide or fragment thereof has a pI that is intrinsically determined by its unique amino acid sequence (which pI can be estimated by the skilled artisan using any of the computer programs designed to predict pI values currently available, calculated using any well-known amino acid pKa table, or measured empirically). Therefore these polypeptides and fragments thereof can serve as specific markers to assist in the determination of the isbelectric point of an unknown protein, polypeptide, or fragmented peptide using techniques such as isoelectric focusing. These polypeptide or fragmented peptide markers serve particularly well for the estimation of apparent isoelectric points of unknown proteins that have apparent isoelectric points close to that of the polypeptide or fragmented peptide markers of the invention.

The technique of isoelectric focusing can be further combined with other techniques such as gel electrophoresis to simultaneously separate a protein on the basis of molecular weight and charge. The ability to simultaneously resolve these polypeptide or fragmented peptide markers and the unknown protein under identical conditions allows for increased accuracy in the determination of the apparent isoelectric point of the unknown protein. This is of particular interest in techniques, such as two dimensional electrophoresis (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 76-77, Prentice Hall, 6th ed. (1991)), where the nature of the procedure dictates that any markers should be resolved simultaneously with the unknown protein. In addition, with such methods, these polypeptides and fragmented peptides thereof can assist in the determination of both the isoelectric point and molecular weight of an unknown protein or fragmented peptide.

[illegible] molecular weight markers of the invention can be visualized using antibodies [illegible]

detection is performed under conventional conditions that do not result in the detection of the unknown protein. It is understood that it may not be possible to generate antibodies against all polypeptide fragments of the invention, since small peptides may not contain immunogenic epitopes. It is further understood that not all antibodies will work in this assay; however, those antibodies which are able to bind polypeptides and fragments of the invention can be readily determined using conventional techniques.

The unknown protein is also visualized by using a conventional staining procedure. The molar excess of unknown protein to polypeptide or fragmented peptide molecular weight markers of the invention is such that the conventional staining procedure predominantly detects the unknown protein. The level of these polypeptide or fragmented peptide molecular weight markers is such as to allow little or no detection of these markers by the conventional staining method. The preferred molar excess of unknown protein to polypeptide molecular weight markers of the invention is between 2 and 100,000 fold. More preferably, the preferred molar excess of unknown protein to these polypeptide molecular weight markers is between 10 and 10,000 fold and especially between 100 and 1,000 fold.

It is understood of course that many techniques can be used for the determination and detection of molecular weight and isoelectric point of an unknown protein, polypeptides, and fragmented peptides thereof using these polypeptide molecular weight markers and peptide fragments thereof and that these embodiments in no way limit the scope of the invention.

In another embodiment, the analysis of the progressive fragmentation of the polypeptides of the invention into specific peptides (D. W. Cleveland et al., *J. Biol. Chem.* 252:1102-1106 (1977)), such as by altering the time or temperature of the fragmentation reaction, can be used as a control for the extent of cleavage of an unknown protein. For example, cleavage of the same amount of polypeptide and unknown protein under identical conditions can allow for a direct comparison of th

As to the specific use of the polypeptides and fragmented peptides of the

ID NOs:4-6 and 12-16 with cyanogen bromide in the absence of glycosylation generates a unique set of fragmented peptide molecular weight markers with molecular weights as set forth in Table 1 on the following page.

Table 1. Molecular Weights of Peptide Fragments Generated by Cyanogen Bromide Digest

SEQ ID NO:4	SEQ ID NO:5	SEQ ID NO:6	SEQ ID NO:12	SEQ ID NO:13	SEQ ID NO:14	SEQ ID NO:15	SEQ ID NO:16
149.2	149.2	374.3	149.2	149.2	149.2	149.2	149.2
4,067.5	1,461.7	701.8	277.4	277.4	277.4	374.5	374.5
	3,960.4	1,154.0	596.7	596.7	596.7	701.8	701.8
	8,420.6	1,196.0	994.1	994.1	970.1	1,154.3	1,154.3
		1,724.0	1,106.2	1,201.3	994.1	1,174.3	1,174.3
		2,040.0	1,201.3	1,212.4	1,106.2	1,196.3	1,196.3
		3,614.0	1,212.4	1,465.8	1,201.3	1,757.0	1,757.0
		4,180.0	1,465.8	1,830.0	1,212.4	2,040.3	2,056.3
		5,327.0	1,830.0	1,908.1	1,465.8	2,330.9	2,330.9
		7,446.0	1,908.1	1,932.1	1,830.0	3,614.5	3,614.5
		7,603.0	2,006.3	2,218.6	1,908.1	4,179.8	4,179.8
		7,611.0	2,218.6	2,673.0	1,946.2	5,327.1	5,327.1
		15,692.0	2,673.0	3,657.1	1,960.2	6,065.8	6,065.8
			4,738.5	4,738.5	2,218.6	6,380.3	6,380.3
			12,088.8	12,088.8	2,673.0	7,446.3	6,487.2
			12,649.5	12,649.5	2,982.3	7,610.5	7,610.5
			16,801.8	16,801.8	4,738.5	10,741.4	10,741.4
			23,353.2	23,353.2	12,088.8	11,292.9	11,302.9
					12,649.5	15,692.4	15,692.4
					16,801.8		

in each peptide and the unique amino acid composition of each peptide determines its

determining molecular weight over the range of the molecular weights of the fragment.

In addition, the preferred purified polypeptides of the invention (SEQ ID NOs:4-6 and 12-16) have calculated molecular weights of approximately 4,199; 13,938; 55,209; 86,983; 89459; 92,781; 88,923; and 87,990 Daltons, respectively. Thus, where an intact protein is used, the use of these polypeptide molecular weight markers allows increased accuracy in the determination of apparent molecular weight of proteins that have apparent molecular weights close to these weights.

Finally, as to the kits that are encompassed by the invention, the constituents of such kits can be varied, but typically contain the polypeptide and fragmented peptide molecular weight markers. Also, such kits can contain the polypeptides wherein a site necessary for fragmentation has been removed. Furthermore, the kits can contain reagents for the specific cleavage of the polypeptide and the unknown protein by chemical or enzymatic cleavage. Kits can further contain antibodies directed against polypeptides or fragments thereof of the invention.

Identification of Unknown Proteins

As set forth above, a polypeptide or peptide fingerprint can be entered into or compared to a database of known proteins to assist in the identification of the unknown protein using mass spectrometry (W.J. Henzel et al., *Proc. Natl. Acad. Sci. USA*, 90:5011-5015 (1993); D. Fenyo et al., *Electrophoresis*, 19:998-1005 (1998)). A variety of computer software programs to facilitate these comparisons are accessible via the Internet, such as Protein Prospector (Internet site: prospector.ucsf.edu), MultiIdent (Internet site: www.expasy.ch/sprot/multiident.html), PeptideSearch (Internet site:www.mann.embl-heiedelberg.de...deSearch/FR_PeptideSearch Form.html), and ProFound (Internet site:www.chait-sgi.rockefeller.edu/cgi-bin/prot-id-frag.html). These programs allow the user to specify the cleavage agent and

weights derived from sequence databases are used in determining the identity of the unknown protein.

In addition, a polypeptide or peptide digest can be sequenced using tandem mass spectrometry (MS/MS) and the resulting sequence searched against databases (J.K. Eng, et al., *J. Am. Soc. Mass Spec.*, 5:976-989 (1994); M. Mann and M. Wilm, *Anal. Chem.*, 66:4390-4399 (1994); J.A. Taylor and R.S. Johnson, *Rapid Comm. Mass Spec.*, 11:1067-1075 (1997)). Searching programs that can be used in this process exist on the Internet, such as Lutefisk 97 (Internet site: www.lsbc.com:70/Lutefisk97.html), and the Protein Prospector, Peptide Search and ProFound programs described above.

Therefore, adding the sequence of a gene and its predicted protein sequence and peptide fragments to a sequence database can aid in the identification of unknown proteins using mass spectrometry.

Antibodies

Antibodies that are immunoreactive with the polypeptides of the invention are provided herein. Such antibodies specifically bind to the polypeptides via the antigen-binding sites of the antibody (as opposed to non-specific binding). Thus, the polypeptides, fragments, variants, fusion proteins, etc., as set forth above may be employed as "immunogens" in producing antibodies immunoreactive therewith. More specifically, the polypeptides, fragment, variants, fusion proteins, etc. contain antigenic determinants or epitopes that elicit the formation of antibodies.

These antigenic determinants or epitopes can be either linear or conformational (discontinuous). Linear epitopes are composed of a single section of amino acids of the polypeptide, while conformational or discontinuous epitopes are composed of amino acids sections from different regions of the polypeptide chain that are brought into close proximity upon protein folding (C. A. Janeway, Jr. and P. Travers, *Immuno Biology* 3:9, Garland Publishing Inc., 2nd ed. (1996)). Because folded proteins have complex surfaces, the number of epitopes available is quite

Publishing Inc., 2nd ed. (1996)). Epitopes may be identified by any of the methods

Thus, one aspect of the present invention relates to the antigenic epitopes of the polypeptides of the invention. Such epitopes are useful for raising antibodies, in particular monoclonal antibodies, as described in more detail below. Additionally, epitopes from the polypeptides of the invention can be used as research reagents, in assays, and to purify specific binding antibodies from substances such as polyclonal sera or supernatants from cultured hybridomas. Such epitopes or variants thereof can be produced using techniques well known in the art such as solid-phase synthesis, chemical or enzymatic cleavage of a polypeptide, or using recombinant DNA technology.

As to the antibodies that can be elicited by the epitopes of the polypeptides of the invention, whether the epitopes have been isolated or remain part of the polypeptides, both polyclonal and monoclonal antibodies may be prepared by conventional techniques. See, for example, *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet et al. (eds.), Plenum Press, New York (1980); and *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1988).

Hybridoma cell lines that produce monoclonal antibodies specific for the polypeptides of the invention are also contemplated herein. Such hybridomas may be produced and identified by conventional techniques. One method for producing such a hybridoma cell line comprises immunizing an animal with a polypeptide; harvesting spleen cells from the immunized animal; fusing said spleen cells to a myeloma cell line, thereby generating hybridoma cells; and identifying a hybridoma cell line that produces a monoclonal antibody that binds the polypeptide. The monoclonal antibodies may be recovered by conventional techniques.

The monoclonal antibodies of the present invention include chimeric antibodies, e.g., humanized versions of murine monoclonal antibodies. Such humanized antibodies may be prepared by known techniques and offer the advantage

derived from a human antibody. Alternatively, a humanized antibody fragment may

region fragment (lacking the antigen-binding site) derived from a human antibody. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann et al., *Nature, 332*:323 (1988); Liu et al., *PNAS, 84*:3439 (1987); Larrick et al., *Bio/Technology, 7*:934 (1989), and Winter and Harris, *TIPS, 14*:139 (May 1993). Procedures to generate antibodies transgenically can be found in GB 2,272,440, US Patent Nos. 5,569,825 and 5,545,806 and related patents claiming priority therefrom, all of which are incorporated by reference herein.

Antigen-binding fragments of the antibodies, which may be produced by conventional techniques, are also encompassed by the present invention. Examples of such fragments include, but are not limited to, Fab and $F(ab')_2$ fragments. Antibody fragments and derivatives produced by genetic engineering techniques are also provided.

In one embodiment, the antibodies are specific for the polypeptides of the present invention and do not cross-react with other proteins. Screening procedures by which such antibodies may be identified are well known, and may involve immunoaffinity chromatography, for example.

Uses Thereof

The antibodies of the invention can be used in assays to detect the presence of the polypeptides or fragments of the invention, either *in vitro* or *in vivo*. The antibodies also may be employed in purifying polypeptides or fragments of the invention by immunoaffinity chromatography.

Those antibodies that additionally can block binding of the polypeptides of the invention to the binding partner may be used to inhibit a biological activity that results from such binding. Such blocking antibodies may be identified using any suitable assay procedure, such as by testing antibodies for the ability to inhibit binding of

results from binding of SVPH to target cells. Antibodies may be assayed for the ability to inhibit SVPH-mediated cell lysis, for example.

Such an antibody may be employed in an *in vitro* procedure, or administered *in vivo* to inhibit a biological activity mediated by the entity that generated the antibody. Disorders caused or exacerbated (directly or indirectly) by the interaction of SVPH with cell surface binding partner thus may be treated. A therapeutic method involves *in vivo* administration of a blocking antibody to a mammal in an amount effective in inhibiting an SVPH-binding partner-mediated biological activity. Monoclonal antibodies are generally preferred for use in such therapeutic methods. In one embodiment, an antigen-binding antibody fragment is employed.

Antibodies may be screened for agonistic (*i.e.*, ligand-mimicking) properties. Such antibodies, upon binding to cell surface binging partner, induce biological effects (e.g., transduction of biological signals) similar to the biological effects induced when SVPH binds to cell surface binding partner.

Compositions comprising an antibody that is directed against SVPH or SVPH binding partner, and a physiologically acceptable diluent, excipient, or carrier, are provided herein. Suitable components of such compositions are as described above for compositions containing SVPH or SVPH binding partner proteins.

Also provided herein are conjugates comprising a detectable (e.g., diagnostic) or therapeutic agent, attached to the antibody.

The following examples are provided to further illustrate particular embodiments of the invention, and are not to be construed as limiting the scope of the present invention.

EXAMPLE 1: Isolation of SVPH Nucleic Acids

A search of the GenBank DNA sequence database revealed two ESTs that share homology with ADAM20 and ADAM21. X85598 showed similarity to the Cys-rich region of ADAM20, while AI214466 showed similarity to the same region in ADAM21. Both ESTs were derived from testis mRNA.

X85598 and AI214466 were used to design primers which were used to

83:3223-3227 (1986)), at 42 C and washing at 42 C in 2x SSC 0.1% SDS using ^{32}P-

labeled deoxyoligonucleotides (5'-CACCTAAGGTGTTCAATTCTTTG-3' (SEQ ID NO:17), 5'-CAAATACTGCAAGTGAGACTTGC-3' (SEQ ID NO:18), 5'-TGCACAACTACGTGTGGTGTACCC-3' (SEQ ID NO:19), and 5'-GAGCCACTGCAATTGAAAAAGTGCCC-3' (SEQ ID NO:20). SVPH-4 clones were isolated under the same conditions using ^{32}P-labeled deoxyoligonucleotides (AATGATGCTCTTGCATGGTCG (SEQ ID NO:21), CTTTCACGGAGCCCATGTAGTTGCAG (SEQ ID NO:22), and TGAAGGAGAAAACGCGCAGATGTCGG (SEQ ID NO:23). DNAs from positively hybridizing phages were purified and characterized by restriction endonuclease mapping, Southern blot analysis, and DNA sequencing.

EXAMPLE 2: DNA Sequence Analysis of SVPH

SVPH-1c has an open reading frame of 820 amino acids (GenBank accession number AF171929) that encodes all of the ADAMs domains, inluding a signal sequence, pro-domain with a Cys switch, catalytic domain with a zinc-binding motif and a Met-turn, disintegrin domain, cysteine-rich domain, a transmembrane domain, and a cytoplasmic domain. However, SVPH-1c (as well as SVPH-1a and SVPH-1b) has a His residue (His 333) instead of a Glu residue in the zinc-binding motif that may affect catalytic activity. The Glu residue binds a water molecule via hydrogen binding and is required for enzymatic activity (Stocker, W. et al., *Protein Sci.*, 4:823-840 (1995)). SVPH-1a and SVPH-1b represent alternative forms of SVPH-1c with differences in the cytoplasmic domain. SVPH-1a has a deletion of 54 amino acids resulting in a protein of 766 amino acids (GenBank accession number AF171930), while SVPH-1b has a divergent 38 amino acid C-terminus resulting in a protein with 787 amino acids (GenBank accession number AF171931). These three forms of SVPH-1 encode cytoplasmic domains of 121, 67, and 88 amino acids, respectively. An unusual feature of the cytoplasmic domain of SVPH-1c is the sequence

SVPH-4a has an open reading frame of [illegible] amino acids (GenBank accession number AF171932) with all of the domains found in ADAMs. Unlike the SVPH-1

catalytic domain. One cDNA, presumably from an alternative RNA splicing event, deletes nine amino acids in the cytoplasmic domain and has been designated SVPH-4b (GenBank accession number AF171933). Interestingly, SVPH-4a and SVPH-4b contain a repeat sequence, QEESK(T/A)KTG (SEQ ID NO:33), in the cytoplasmic domain, which was not found in GenBank.

As noted above, SVPH-1a, SVPH-1b, and SVPH-1c diverge from the consensus zinc-binding cluster (**HE**XX**H**XX**G**XX**HD**) (SEQ ID NO:31) in the catalytic domain with a Glu to His change at position 333. To analyze these proteins further, DNA and protein sequence multiple alignments of all known mammalian ADAMs (http://www.med.virginia.edu/~jag6n/adams.html) were produced using the PILEUP program from the Wisconsin Package (Wisconsin Package 10.1, Genetics Computer Group, Madison, WI). Protein multiple alignments were generated using the modified PAM scoring matrix of Gribskov and Burgess (Gribskov, M. et al., *Nucleic Acids Res.*, 14:6745-6763 (1986)) provided in the Wisconsin Package, with gap-open and gap-extend penalties of 30 and 1, respectively. Nucleic acid multiple alignments were generated using a scoring matrix with A, C, G, T matches scoring unity, mismatches scoring zero, and gap-open and gap-extend penalties of 5 and 1 respectively. Unrooted maximum parsimony trees were estimated by the Wisconsin Package implementation of PAUP (version 4.0), starting from multiple alignments produced by PILEUP. PAUP parameters were set to use accelerated transformation character-state optimization with unordered, equally weighted characters.

This alignment was used to infer a maximum parsimony phylogeny (Fig. 2). Due to the large number of taxa involved, the phylogeny was inferred using a heuristic tree search, which does not perform an exhaustive search of all possible tree topologies. Examination of the phylogenetic tree revealed an interesting pattern with respect to the presence of a zinc-binding motif. The ADAM sequences can be divided into two well-separated regions of the phylogeny, as marked by the arrow in Fig. 2. ADAM family members that contain a consensus zinc-binding site

(ADAMs 4, 6, 7, 11, 22, 23, and SVPH-1) presumably arose from a catalytically

example, ADAM4, ADAM7, and SVPH-1 all possess the three His residues and the Asp after the third conserved His. Finally, the corresponding region in ADAMs 2, 3, 5, 18 and 27 is quite distinct. As these sequences form clusters quite divergent from the zinc-binding site-containing ADAMs, it is most likely that the zinc-binding site arose once in the common ancestor to the ADAMs and was lost in those lineages which do not possess a zinc-binding site (denoted by an 'X' in Fig. 2).

EXAMPLE 3: Chromosome Mapping of SVPH

Radiation hybrid mapping (Walter, et al., *Nat. Genet.*, 7: 22-28 (1994)) was done using the GeneBridge 4 radiation-hybrid mapping panel (Research Genetics, Huntsville, AL). The panel was screened with specific primer pairs for SVPH-1 (sense: 5'-TCGATAATGCATGAAGGCAACCCACC-3' (SEQ ID NO:24) and antisense: 5'-CAAGTCTCACTTGCAGTATTTGCGCC-3' (SEQ ID NO:25), and SVPH-4 (sense; 5'-GCCACTGCATGTATGGGTG-3' (SEQ ID NO:26) and antisense: 5'-GACACTCTTTGCTTTGGGTCG-3' (SEQ ID NO:27) which generated products of 298 and 263 bp, respectively. PCR products were subjected to Southern blot analysis using an internal oligonucleotide probe specific for each gene. Data from two independent PCR screenings for each primer pair were scored against STS markers from the Whitehead Institute/MIT Center for Genome Research database using the statistical program RHMAPPER. LOD scores were >3.0 in all cases.

SVPH-1a, SVPH-1b, and SVPH-1c were mapped to chromosome 4q34, 1.51 cR distal from AFM312WG1. The sequential order of known markers relative to SVPH-1 on the Whitehead framework map was D4S1545, PDGH (Hydroxyprostaglandin Dehyrogenase 15)/SVPH-1/WI-21773/GPM6A (Glycoprotein M6A). This region is syntenic to mouse chromosome 8. SVPH-4a and SVPH-4b were mapped to chromosome 1p11-13, 1.65 cR distal to D1S453. The sequential order of markers relative to SVPH-4 on the Whitehead framework map was CD2

EXAMPLE 4: Tissue Distribution of SVPH

Northern blot analysis was used to determine the tissue distribution of SVPH-1 and SVPH-4. Northern blots were purchased from Clontech (catalog number 7760-1, 7759-1, 7755-1, 7750-1). Each lane contained approximately 2 μg of the indicated poly A$^+$ RNA. The blots were treated with Stark's buffer (50% formamide, 50mM KPO_4, 5 x SSC, 1% SDS, 5X Denhardt's, 0.05% sarcosyl, 300mg/ml salmon sperm DNA) at 63°C for at least 1h and then probed with ^{32}P-labeled riboprobes in Stark's buffer at 63°C, overnight (Cosman et al., *Nature*, 312:768-771 (1984)). Blots were then sequentially washed to high stringency (0.1 x SSC, 0.1% SDS, 63°C) and exposed to film. Films were developed in an automated x-ray film processor. SVPH-1 (nt 1068 to 1786 of SEQ ID NOs:7-9) and SVPH-4 (nt 1343 to 1779 of SEQ ID NOs:10-11) anti-sense riboprobes were prepared by *in vitro* transcription from a T7 RNA promoter with a commercially available kit (MAXIscript, Ambion, Inc., Austin, TX) using [α-^{32}P]-UTP as the labeled nucleotide.

As indicated in Figure 1, both SVPH-1 and SVPH-4 were specifically expressed in testes with a single mRNA species of approximately 3.0 kb. No signals were detected in the other RNA samples.

EXAMPLE 5: Monoclonal Antibodies

This example illustrates a method for preparing monoclonal antibodies that bind an SVPH-1, SVPH-1a, SVPH-1b, SVPH-1c, SVPH-4, SVPH-4a or SVPH-4b polypeptide. Suitable immunogens that may be employed in generating such antibodies include, but are not limited to, purified SVPH-1, SVPH-1a, SVPH-1b, SVPH-1c, SVPH-4, SVPH-4a or SVPH-4b polypeptide or an immunogenic fragment thereof such as the extracellular domain, or fusion proteins containing SVPH-1, SVPH-1a, SVPH-1b, SVPH-1c, SVPH-4, SVPH-4a or SVPH-4b (e.g., a soluble SVPH-1 Fc fusion protein).

[illegible]

Briefly, mice are immunized with SVPH-1, SVPH-1a, SVPH-1b, SVPH-1c, SVPH-4, [illegible]

injected in amounts ranging from 10-100 µg subcutaneously or intraperitoneally. Ten to twelve days later, the immunized animals are boosted with additional immunogen emulsified in incomplete Freund's adjuvant. Mice are periodically boosted thereafter on a weekly to bi-weekly immunization schedule. Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision to test for SVPH-1, SVPH-1a, SVPH-1b, SVPH-1c, SVPH-4, SVPH-4a or SVPH-4b antibodies by dot blot assay, ELISA (Enzyme-Linked Immunosorbent Assay) or inhibition of binding partner binding.

Following detection of an appropriate antibody titer, positive animals are provided one last intravenous injection of SVPH-1, SVPH-1a, SVPH-1b, SVPH-1c, SVPH-4, SVPH-4a or SVPH-4b in saline. Three to four days later, the animals are sacrificed, spleen cells harvested, and spleen cells are fused to a murine myeloma cell line, e.g., NS1 or preferably P3x63Ag8.653 (ATCC CRL 1580). Fusions generate hybridoma cells, which are plated in multiple microtiter plates in a HAT (hypoxanthine, aminopterin and thymidine) selective medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells are screened by ELISA for reactivity against purified SVPH-1, SVPH-1a, SVPH-1b, SVPH-1c, SVPH-4, SVPH-4a or SVPH-4b by adaptations of the techniques disclosed in Engvall et al., *Immunochem.* 8:871, 1971 and in U.S. Patent 4,703,004. A preferred screening technique is the antibody capture technique described in Beckmann et al., *J. Immunol.* 144:4212, (1990). Positive hybridoma cells can be injected intraperitoneally into syngeneic BALB c mice to produce ascites containing high concentrations of anti-SVPH-1, SVPH-1a, SVPH-1b, SVPH-1c, SVPH-4, SVPH-4a or SVPH-4b monoclonal antibodies. Alternatively, hybridoma cells can be grown *in vitro* in flasks or roller bottles by various techniques. Monoclonal antibodies produced in mouse ascites can be purified by ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively,

EXAMPLE 6: Binding Assay

Full length SVPH-1, SVPH-1a, SVPH-1b, SVPH-1c, SVPH-4, SVPH-4a or SVPH-4b is expressed and tested for the ability to bind its binding partner. The binding assay is conducted as follows.

A fusion protein comprising a leucine zipper peptide fused to the N-terminus of a soluble binding partner polypeptide (LZ-binding partner) is employed in the assay. An expression construct is prepared, essentially as described for preparation of the FLAG®-binding partner expression construct in Wiley et al., *Immunity*, 3:673-682, (1995), which is hereby incorporated by reference, except that DNA encoding the FLAG® peptide is replaced with a sequence encoding a modified leucine zipper that allows for trimerization. The construct, in expression vector pDC409, encodes a leader sequence derived from human cytomegalovirus, followed by the leucine zipper moiety fused to the N-terminus of a soluble binding partner polypeptide. The LZ-binding partner is expressed in CHO cells, and can be purified from the culture supernatant.

The expression vector designated pDC409 is a mammalian expression vector derived from the pDC406 vector described in McMahan et al., *EMBO J.* 10:2821-2832, (1991), which is hereby incorporated by reference. Features added to pDC409 (compared to pDC406) include additional unique restriction sites in the multiple cloning site (mcs); three stop codons (one in each reading frame) positioned downstream of the mcs; and a T7 polymerase promoter, downstream of the mcs, that facilitates sequencing of DNA inserted into the mcs.

For expression of full length human SVPH-1, SVPH-1a, SVPH-1b, SVPH-1c, SVPH-4, SVPH-4a or SVPH-4b protein, the entire coding region (*i.e.*, the DNA sequence presented in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11) is amplified by polymerase chain reaction (PCR). The isolated and amplified DNA is inserted into the expression

[illegible]

[illegible]

or SVPH-4b polypeptide, as discussed above. Cells are cultured in DMEM

[illegible]

48 hours after transfection, cells are detached non-enzymatically and incubated with LZ-binding partner (5 mg/ml), a biotinylated anti-LZ monoclonal antibody (5 mg/ml), and phycoerythrin-conjugated streptavidin (1:400), before analysis by fluorescence-activated cell scanning (FACS). The cytometric analysis is conducted on a FACscan (Beckton Dickinson, San Jose, CA).

The cells expressing LZ-binding partner will show significantly enhanced binding of SVPH-1, SVPH-1a, SVPH-1b, SVPH-1c, SVPH-4, SVPH-4a or SVPH-4b, compared to the control cells not expressing LZ-binding partner.

The specification is most thoroughly understood in light of the teachings of the references cited within the specification which are hereby incorporated by reference. The embodiments within the specification provide an illustration of embodiments of the invention and should not be construed to limit the scope of the invention. The skilled artisan readily recognizes that many other embodiments are encompassed by the invention.

What is claimed is:

1. An isolated SVPH nucleic acid molecule selected from the group consisting of:

(a) the DNA sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9;

(b) an isolated nucleic acid molecule encoding an amino acid sequence comprising the sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14;

(c) an isolated nucleic acid molecule that hybridizes to either strand of a denatured, double-stranded DNA comprising the nucleic acid sequence of (a) or (b) under conditions of moderate stringency in 50% formamide and 6XSSC, at 42°C with washing conditions of 60°C, 0.5XSSC, 0.1% SDS;

(d) an isolated nucleic acid molecule derived by *in vitro* mutagenesis from SEQ ID NO:1, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9;

(e) an isolated nucleic acid molecule degenerate from SEQ ID NO:1, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9 as a result of the genetic code; and

(f) an isolated nucleic acid molecule selected from the group consisting of human SVPH 1 DNA; an allelic variant of human SVPH 1 DNA; and a species homolog of SVPH 1 DNA.

2. The nucleic acid molecule of claim 1 selected from the group consisting of SEQ ID NO:1, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9.

3. A recombinant vector that directs the expression of the nucleic acid molecule of claim 1.

4. An isolated polypeptide encoded by the nucleic acid molecule of claim 1.

5. An isolated polypeptide according to claim 4 having a molecular weight selected from the group consisting of approximately 4,199; 86,983; 89,459; and 102,781 Daltons as determined by SDS-PAGE.

8. Isolated antibodies according to claim 7, wherein the antibodies are

9. A host cell transfected or transduced with the vector of claim 3.

10. A method for the production of SVPH 1 polypeptide comprising culturing a host cell of claim 9 under conditions promoting expression, and recovering the polypeptide from the culture medium.

11. The method of claim 10, wherein the host cell is selected from the group consisting of bacterial cells, yeast cells, plant cells, and animal cells.

12. The method of claim 10, wherein the host cell is a mammalian cell.

13. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14.

14. An oligomer comprising a polypeptide of claim 4.

15. An isolated SVPH nucleic acid molecule selected from the group consisting of:

(a) the DNA sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:10, and SEQ ID NO:11;

(b) an isolated nucleic acid molecule encoding an amino acid sequence comprising the sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:15, and SEQ ID NO:16;

(c) an isolated nucleic acid molecule that hybridizes to either strand of a denatured, double-stranded DNA comprising the nucleic acid sequence of (a) or (b) under conditions of moderate stringency in 50% formamide and 6XSSC, at 42° C with washing conditions of 60°C, 0.5XSSC, 0.1% SDS;

(d) an isolated nucleic acid molecule derived by *in vitro* mutagenesis from SEQ ID NO:3, SEQ ID NO:10, and SEQ ID NO:11;

(e) an isolated nucleic acid molecule degenerate from SEQ ID NO:3, SEQ ID NO:10, and SEQ ID NO:11 as a result of the genetic code; and

(f) an isolated nucleic acid molecule selected from the group consisting of human SVPH 1 DNA, an allelic variant of human SVPH 1 DNA, and a species

16. The nucleic acid molecule of claim 15 selected from the group consisting of SEQ ID NO:3, SEQ ID NO:10, and SEQ ID NO:11.

17. A recombinant vector that directs the expression of the nucleic acid molecule of claim 15.

18. An isolated polypeptide encoded by the nucleic acid molecule of claim 15.

19. An isolated polypeptide according to claim 18 having a molecular weight selected from the group consisting of approximately 55,209; 88,923; and 87,990 Daltons as determined by SDS-PAGE.

20. An isolated polypeptide according to claim 18 in non-glycosylated form.

21. Isolated antibodies that bind to a polypeptide of claim 18.

22. Isolated antibodies according to claim 21, wherein the antibodies are monoclonal antibodies.

23. A host cell transfected or transduced with the vector of claim 17.

24. A method for the production of SVPH 4 polypeptide comprising culturing a host cell of claim 23 under conditions promoting expression, and recovering the polypeptide from the culture medium.

25. The method of claim 24, wherein the host cell is selected from the group consisting of bacterial cells, yeast cells, plant cells, and animal cells.

26. The method of claim 24, wherein the host cell is a mammalian cell.

27. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:15, and SEQ ID NO:16.

28. An oligomer comprising a polypeptide of claim 18.

29. An isolated SVPH nucleic acid molecule selected from the group consisting of:

(a) the DNA sequence of SEQ ID NO:2;

(b) an isolated nucleic acid molecule encoding an amino acid sequence

a denatured, double-stranded DNA comprising the nucleic acid sequence of (a) or (b)

under conditions of moderate stringency in 50% formamide and 6XSSC, at 42° C with washing conditions of 60° C, 0.5XSSC, 0.1% SDS;

(d) an isolated nucleic acid molecule derived by *in vitro* mutagenesis from SEQ ID NO:2;

(e) an isolated nucleic acid molecule degenerate from SEQ ID NO:2 as a result of the genetic code; and

(f) an isolated nucleic acid molecule selected from the group consisting of human SVPH 3 DNA; an allelic variant of human SVPH 3 DNA; and a species homolog of SVPH 3 DNA.

30. The nucleic acid molecule of claim 29, wherein the DNA sequence comprises SEQ ID NO:2.

31. A recombinant vector that directs the expression of the nucleic acid molecule of claim 29.

32. An isolated polypeptide encoded by the nucleic acid molecule of claim 29.

33. An isolated polypeptide according to claim 32 having a molecular weight of approximately 13,938 Daltons as determined by SDS-PAGE.

34. An isolated polypeptide according to claim 32 in non-glycosylated form.

35. Isolated antibodies that bind to a polypeptide of claim 32.

36. Isolated antibodies according to claim 35, wherein the antibodies are monoclonal antibodies.

37. A host cell transfected or transduced with the vector of claim 31.

38. A method for the production of SVPH 3 polypeptide comprising culturing a host cell of claim 37 under conditions promoting expression, and recovering the polypeptide from the culture medium.

39. The method of claim 38, wherein the host cell is selected from the [illegible] cells, yeast cells, plant cells, and animal cells.

[illegible] NO:5.

[illegible] polypeptide of claim 32

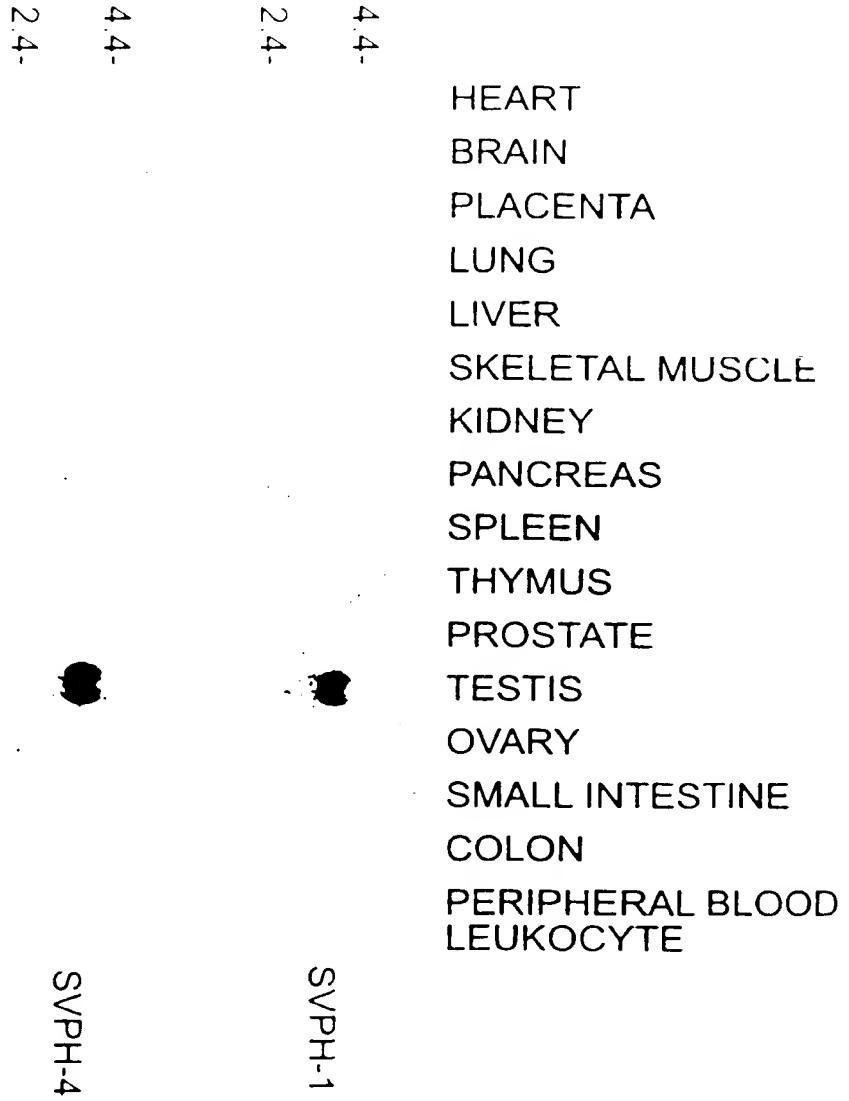
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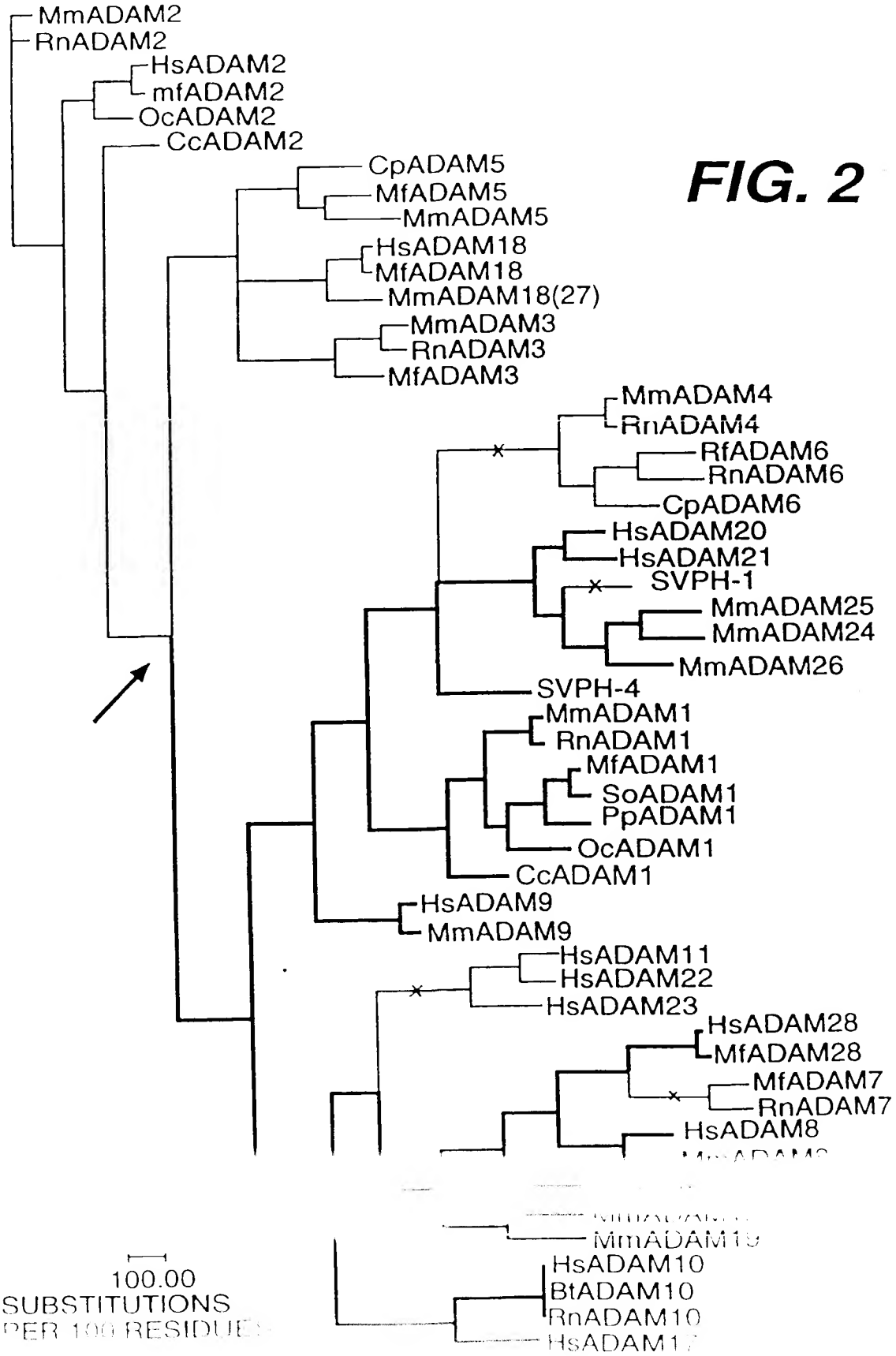
FIG 1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SUBSTITUTIONS

FIG. 2

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may be any amino acid

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Asn Cys Ser Tyr Ile Ser Phe Phe Lys His Ile Ser Ser Gly Ala Thr
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<212> DNA
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<211> 2346
<212> DNA
<213> Homo sapiens

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<212> PRT
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Ser Gly His Ile Gln Asp Glu His Pro Gln Tyr His Ser Pro Pro Asp
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Val Val Ile Pro Val Arg Ile Thr Gly Thr Thr Arg Gly Met Thr Pro
35 40 45

Pro Gly Trp Leu Ser Tyr Ile Leu Pro Phe Gly Gly Gln Lys His Ile
50 55 60

Ile His Ile Lys Val Lys Lys Leu Leu Phe Ser Lys His Leu Pro Val
65 70 75 80

Phe Thr Tyr Thr Asp Gln Gly Ala Ile Leu Glu Asp Gln Pro Phe Val
85 90 95

Gln Asn Asn Cys Tyr Tyr His Gly Tyr Val Glu Gly Asp Pro Gln Ser
100 105 110

Leu Val Ser Leu Ser Thr Cys Phe Gly Gly Phe Gln Gly Ile Leu Gln
115 120 125

Ile Asn Asp Phe Ala Tyr Glu Ile Lys Pro Leu Ala Phe Ser Thr Thr
130 135 140

Phe Glu Glu Ile Asp Asn Ser Thr Gln Lys Gln Ser Ser Tyr Val Gly
180 185 190

Trp Trp Ile His Phe Arg Ile Val Glu Ile Val Val Val Ile Asp Asn
195 200 205

Tyr Leu Tyr Ile Arg Tyr Glu Arg Asn Asp Ser Lys Leu Leu Glu Asp
210 215 220

Leu Tyr Val Ile Val Asn Ile Val Asp Ser Ile Leu Asp Val Ile Gly
225 230 235 240

Val Lys Val Leu Leu Phe Gly Leu Glu Ile Trp Thr Asn Lys Asn Leu
245 250 255

Ile Val Val Asp Asp Val Arg Lys Ser Val His Leu Tyr Cys Lys Trp
260 265 270

Lys Ser Glu Asn Ile Thr Pro Arg Met Gln His Asp Thr Ser His Leu
275 280 285

Phe Thr Thr Leu Gly Leu Arg Gly Leu Ser Gly Ile Gly Ala Phe Arg
290 295 300

Gly Met Cys Thr Pro His Arg Ser Cys Ala Ile Val Thr Phe Met Asn
305 310 315 320

Lys Thr Leu Gly Thr Phe Ser Ile Ala Val Ala His His Leu Gly His
325 330 335

Asn Leu Gly Met Asn His Asp Glu Asp Thr Cys Arg Cys Ser Gln Pro
340 345 350

Arg Cys Ile Met His Glu Gly Asn Pro Pro Ile Thr Lys Phe Ser Asn
355 360 365

Cys Ser Tyr Gly Asp Phe Trp Glu Tyr Thr Val Glu Arg Thr Lys Cys
370 375 380

Leu Leu Glu Thr Val His Thr Lys Asp Ile Phe Asn Val Lys Arg Cys
385 390 395 400

Gly Asn Gly Val Val Glu Glu Gly Glu Glu Cys Asp Cys Gly Pro Leu
405 410 415

Lys His Cys Ala Lys Asp Pro Cys Cys Leu Ser Asn Cys Thr Leu Thr
420 425 430

Asp Gly Ser Thr Cys Ala Phe Gly Leu Cys Cys Lys Asp Cys Lys Phe
435 440 445

Leu Pro Ser Gly Lys Val Cys Arg Lys Glu Val Asn Glu Cys Asp Leu

Val Glu Asp Gly Ile Pro Cys Lys Glu Arg Gly Tyr Cys Tyr Glu Lys
485 490 495

Ser Cys His Asp Arg Asn Glu Gln Cys Arg Arg Ile Phe Gly Ala Gly
500 505 510

Ala Asn Thr Ala Ser Glu Thr Cys Tyr Lys Glu Leu Asn Thr Leu Gly
515 520 525

Asp Arg Val Gly His Cys Gly Ile Lys Asn Ala Thr Tyr Ile Lys Cys
530 535 540

Asn Ile Ser Asp Val Gln Cys Gly Arg Ile Gln Cys Glu Asn Val Thr
545 550 555 560

Glu Ile Pro Asn Met Ser Asp His Thr Thr Val His Trp Ala Arg Phe
565 570 575

Asn Asp Ile Met Cys Trp Ser Thr Asp Tyr His Leu Gly Met Lys Gly
580 585 590

Pro Asp Ile Gly Glu Val Lys Asp Gly Thr Glu Cys Gly Ile Asp His
595 600 605

Ile Cys Ile His Arg His Cys Val His Ile Thr Ile Leu Asn Ser Asn
610 615 620

Cys Ser Pro Ala Phe Cys Asn Lys Arg Gly Ile Cys Asn Asn Lys His
625 630 635 640

His Cys His Cys Asn Tyr Leu Trp Asp Pro Pro Asn Cys Leu Ile Lys
645 650 655

Gly Tyr Gly Gly Ser Val Asp Ser Gly Pro Pro Pro Lys Arg Lys Lys
660 665 670

Lys Lys Lys Phe Cys Tyr Leu Cys Ile Leu Leu Leu Ile Val Leu Phe
675 680 685

Ile Leu Leu Cys Cys Leu Tyr Arg Leu Cys Lys Lys Ser Lys Pro Ile
690 695 700

Lys Lys Gln Gln Asp Val Gln Thr Pro Ser Ala Lys Glu Glu Glu Lys
705 710 715 720

Ile Gln Arg Arg Pro His Glu Leu Pro Pro Gln Ser Gln Pro Trp Val
725 730 735

Met Pro Ser Gln Ser Gln Pro Pro Val Thr Pro Ser Gln Arg Gln Pro
740 745 750

Gln Leu Met Pro Ser Gln Ser Gln Pro Pro Val Thr Pro Ser
755 760 765

<213> Homo sapiens

<400> 13

Met Lys Met Leu Leu Leu Leu His Cys Leu Gly Val Phe Leu Ser Cys
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Ser Gly His Ile Gln Asp Glu His Pro Gln Tyr His Ser Pro Pro Asp
20 25 30

Val Val Ile Pro Val Arg Ile Thr Gly Thr Thr Arg Gly Met Thr Pro
35 40 45

Pro Gly Trp Leu Ser Tyr Ile Leu Pro Phe Gly Gly Gln Lys His Ile
50 55 60

Ile His Ile Lys Val Lys Lys Leu Leu Phe Ser Lys His Leu Pro Val
65 70 75 80

Phe Thr Tyr Thr Asp Gln Gly Ala Ile Leu Glu Asp Gln Pro Phe Val
85 90 95

Gln Asn Asn Cys Tyr Tyr His Gly Tyr Val Glu Gly Asp Pro Glu Ser
100 105 110

Leu Val Ser Leu Ser Thr Cys Phe Gly Gly Phe Gln Gly Ile Leu Gln
115 120 125

Ile Asn Asp Phe Ala Tyr Glu Ile Lys Pro Leu Ala Phe Ser Thr Thr
130 135 140

Phe Glu His Leu Val Tyr Lys Met Asp Ser Glu Glu Lys Gln Phe Ser
145 150 155 160

Thr Met Arg Ser Gly Phe Met Gln Asn Glu Ile Thr Cys Arg Met Glu
165 170 175

Phe Glu Glu Ile Asp Asn Ser Thr Gln Lys Gln Ser Ser Tyr Val Gly
180 185 190

Trp Trp Ile His Phe Arg Ile Val Glu Ile Val Val Val Ile Asp Asn
195 200 205

Tyr Leu Tyr Ile Arg Tyr Glu Arg Asn Asp Ser Lys Leu Leu Glu Asp
210 215 220

Leu Tyr Val Ile Val Asn Ile Val Asp Ser Ile Leu Asp Val Ile Gly
225 230 235 240

Val Lys Val Leu Leu Phe Gly Leu Glu Ile Trp Thr Asn Lys Asn Leu
245 250 255

Ile Val Val Asp Asp Val Arg Lys Ser Val His Leu Tyr Cys Lys Trp
260 265 270

Phe Thr Thr Leu Gly Leu Arg Gly Leu Ser Gly Ile Gly Ala Phe Arg
290 295 300

Gly Met Cys Thr Pro His Arg Ser Cys Ala Ile Val Thr Phe Met Asn
305 310 315 320

Lys Thr Leu Gly Thr Phe Ser Ile Ala Val Ala His His Leu Gly His
325 330 335

Asn Leu Gly Met Asn His Asp Glu Asp Thr Cys Arg Cys Ser Gln Pro
340 345 350

Arg Cys Ile Met His Glu Gly Asn Pro Pro Ile Thr Lys Phe Ser Asn
355 360 365

Cys Ser Tyr Gly Asp Phe Trp Glu Tyr Thr Val Glu Arg Thr Lys Cys
370 375 380

Leu Leu Glu Thr Val His Thr Lys Asp Ile Phe Asn Val Lys Arg Cys
385 390 395 400

Gly Asn Gly Val Val Glu Glu Gly Glu Glu Cys Asp Cys Gly Pro Leu
405 410 415

Lys His Cys Ala Lys Asp Pro Cys Cys Leu Ser Asn Cys Thr Leu Thr
420 425 430

Asp Gly Ser Thr Cys Ala Phe Gly Leu Cys Cys Lys Asp Cys Lys Phe
435 440 445

Leu Pro Ser Gly Lys Val Cys Arg Lys Glu Val Asn Glu Cys Asp Leu
450 455 460

Pro Glu Trp Cys Asn Gly Thr Ser His Lys Cys Pro Asp Asp Phe Tyr
465 470 475 480

Val Glu Asp Gly Ile Pro Cys Lys Glu Arg Gly Tyr Cys Tyr Glu Lys
485 490 495

Ser Cys His Asp Arg Asn Glu Gln Cys Arg Arg Ile Phe Gly Ala Gly
500 505 510

Ala Asn Thr Ala Ser Glu Thr Cys Tyr Lys Glu Leu Asn Thr Leu Gly
515 520 525

Asp Arg Val Gly His Cys Gly Ile Lys Asn Ala Thr Tyr Ile Lys Cys
530 535 540

Asn Ile Ser Asp Val Gln Cys Gly Arg Ile Gln Cys Glu Asn Val Thr
545 550 555 560

Glu Ile Pro Asn Met Ser Asp His Thr Thr Val His Trp Ala Arg Phe
565 570 575

Pro Asp Ile Gly Glu Val Lys Asp Gly Thr Glu Cys Gly Ile Asp His
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Ile Cys Ile His Arg His Cys Val His Ile Thr Ile Leu Asn Ser Asn
610 615 620

Cys Ser Pro Ala Phe Cys Asn Lys Arg Gly Ile Cys Asn Asn Lys His
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His Cys His Cys Asn Tyr Leu Trp Asp Pro Pro Asn Cys Leu Ile Lys
645 650 655

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660 665 670

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675 680 685

Ile Leu Leu Cys Cys Leu Tyr Arg Leu Cys Lys Lys Ser Lys Pro Ile
690 695 700

Lys Lys Gln Gln Asp Val Gln Thr Pro Ser Ala Lys Glu Glu Glu Lys
705 710 715 720

Ile Gln Arg Arg Pro His Glu Leu Pro Pro Gln Ser Gln Pro Trp Val
725 730 735

Met Pro Ser Gln Ser Gln Pro Pro Val Thr Pro Ser Gln Ser His Pro
740 745 750

Gln Val Met Pro Ser Gln Ser Gln Pro Pro Gln Asn Leu Phe Leu Phe
755 760 765

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770 775 780

Gln Ala Thr
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<211> 820
<212> PRT
<213> Homo sapiens

<400> 14
Met Lys Met Leu Leu Leu Leu His Cys Leu Gly Val Phe Leu Ser Cys
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Ser Gly His Ile Gln Asp Glu His Pro Gln Tyr His Ser Pro Pro Asp
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Val Val Ile Pro Val Arg Ile Thr Gly Thr Thr Arg Gly Met Thr Pro

Ile His Ile Lys Val Lys Lys Leu Leu Phe Ser Lys His Leu Pro Val
65 70 75 80

Phe Thr Tyr Thr Asp Gln Gly Ala Ile Leu Glu Asp Gln Pro Phe Val
85 90 95

Gln Asn Asn Cys Tyr Tyr His Gly Tyr Val Glu Gly Asp Pro Glu Ser
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Leu Val Ser Leu Ser Thr Cys Phe Gly Gly Phe Gln Gly Ile Leu Gln
115 120 125

Ile Asn Asp Phe Ala Tyr Glu Ile Lys Pro Leu Ala Phe Ser Thr Thr
130 135 140

Phe Glu His Leu Val Tyr Lys Met Asp Ser Glu Glu Lys Gln Phe Ser
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Thr Met Arg Ser Gly Phe Met Gln Asn Glu Ile Thr Cys Arg Met Glu
165 170 175

Phe Glu Glu Ile Asp Asn Ser Thr Gln Lys Gln Ser Ser Tyr Val Gly
180 185 190

Trp Trp Ile His Phe Arg Ile Val Glu Ile Val Val Val Ile Asp Asn
195 200 205

Tyr Leu Tyr Ile Arg Tyr Glu Arg Asn Asp Ser Lys Leu Leu Glu Asp
210 215 220

Leu Tyr Val Ile Val Asn Ile Val Asp Ser Ile Leu Asp Val Ile Gly
225 230 235 240

Val Lys Val Leu Leu Phe Gly Leu Glu Ile Trp Thr Asn Lys Asn Leu
245 250 255

Ile Val Val Asp Asp Val Arg Lys Ser Val His Leu Tyr Cys Lys Trp
260 265 270

Lys Ser Glu Asn Ile Thr Pro Arg Met Gln His Asp Thr Ser His Leu
275 280 285

Phe Thr Thr Leu Gly Leu Arg Gly Leu Ser Gly Ile Gly Ala Phe Arg
290 295 300

Gly Met Cys Thr Pro His Arg Ser Cys Ala Ile Val Thr Phe Met Asn
305 310 315 320

Lys Thr Leu Gly Thr Phe Ser Ile Ala Val Ala His His Leu Gly His
325 330 335

Asn Leu Gly Met Asn His Asp Glu Asp Thr Cys Arg Cys Ser Gln Pro
340 345 350

Cys Ser Tyr Gly Asp Phe Trp Glu Tyr Thr Val Glu Arg Thr Lys Cys
370 375 380

Leu Leu Glu Thr Val His Thr Lys Asp Ile Phe Asn Val Lys Arg Cys
385 390 395 400

Gly Asn Gly Val Val Glu Glu Gly Glu Glu Cys Asp Cys Gly Pro Leu
405 410 415

Lys His Cys Ala Lys Asp Pro Cys Cys Leu Ser Asn Cys Thr Leu Thr
420 425 430

Asp Gly Ser Thr Cys Ala Phe Gly Leu Cys Cys Lys Asp Cys Lys Phe
435 440 445

Leu Pro Ser Gly Lys Val Cys Arg Lys Glu Val Asn Glu Cys Asp Leu
450 455 460

Pro Glu Trp Cys Asn Gly Thr Ser His Lys Cys Pro Asp Asp Phe Tyr
465 470 475 480

Val Glu Asp Gly Ile Pro Cys Lys Glu Arg Gly Tyr Cys Tyr Glu Lys
485 490 495

Ser Cys His Asp Arg Asn Glu Gln Cys Arg Arg Ile Phe Gly Ala Gly
500 505 510

Ala Asn Thr Ala Ser Glu Thr Cys Tyr Lys Glu Leu Asn Thr Leu Gly
515 520 525

Asp Arg Val Gly His Cys Gly Ile Lys Asn Ala Thr Tyr Ile Lys Cys
530 535 540

Asn Ile Ser Asp Val Gln Cys Gly Arg Ile Gln Cys Glu Asn Val Thr
545 550 555 560

Glu Ile Pro Asn Met Ser Asp His Thr Thr Val His Trp Ala Arg Phe
565 570 575

Asn Asp Ile Met Cys Trp Ser Thr Asp Tyr His Leu Gly Met Lys Gly
580 585 590

Pro Asp Ile Gly Glu Val Lys Asp Gly Thr Glu Cys Gly Ile Asp His
595 600 605

Ile Cys Ile His Arg His Cys Val His Ile Thr Ile Leu Asn Ser Asn
610 615 620

Cys Ser Pro Ala Phe Cys Asn Lys Arg Gly Ile Cys Asn Asn Lys His
625 630 635 640

His Cys His Cys Asn Tyr Leu Trp Asp Pro Pro Asn Cys Leu Ile Lys
645 650 655

```
Lys Lys Lys Phe Cys Tyr Leu Cys Ile Leu Leu Leu Ile Val Leu Phe
        675                 680                 685

Ile Leu Leu Cys Cys Leu Tyr Arg Leu Cys Lys Lys Ser Lys Pro Ile
    690                 695                 700

Lys Lys Gln Gln Asp Val Gln Thr Pro Ser Ala Lys Glu Glu Glu Lys
705                 710                 715                 720

Ile Gln Arg Arg Pro His Glu Leu Pro Pro Gln Ser Gln Pro Trp Val
                725                 730                 735

Met Pro Ser Gln Ser Gln Pro Pro Val Thr Pro Ser Gln Ser His Pro
            740                 745                 750

Arg Val Met Pro Ser Gln Ser Gln Pro Pro Val Met Pro Ser Gln Ser
        755                 760                 765

His Pro Gln Leu Thr Pro Ser Gln Ser Gln Pro Pro Val Met Pro Ser
    770                 775                 780

Gln Ser His Pro Gln Leu Thr Pro Ser Gln Ser Gln Pro Pro Val Thr
785                 790                 795                 800

Pro Ser Gln Arg Gln Pro Gln Leu Met Pro Ser Gln Ser Gln Pro Pro
                805                 810                 815

Val Thr Pro Ser
            820
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<210> 15
<211> 790
<212> PRT
<213> Homo sapiens

<400> 15

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Met Arg Ser Val Gln Ile Phe Leu Ser Gln Cys Arg Leu Leu Leu Leu
  1               5                  10                  15

Leu Val Pro Thr Met Leu Leu Lys Ser Leu Gly Glu Asp Val Ile Phe
            20                  25                  30

His Pro Gln Gly Glu Phe Asp Ser Tyr Glu Val Thr Ile Pro Glu Lys
        35                  40                  45

Leu Ser Phe Arg Gly Glu Val Gln Gly Val Val Ser Ile Val Ser Tyr
    50                  55                  60

Leu Leu Gln Leu Lys Gly Lys Lys His Val Leu His Leu Trp Pro Lys
65                  70                  75                  80

Arg Leu Leu Leu Pro Arg His Leu Arg Val Phe Ser Ile Thr Glu His
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Met Gly Ser Val Lys Glu Ser Leu Asp Ser Lys Ala Thr Ile Ser Thr
115 120 125

Cys Met Gly Gly Leu Arg Gly Val Phe Asn Ile Asp Ala Lys His Tyr
130 135 140

Gln Ile Glu Pro Leu Lys Ala Ser Pro Ser Phe Glu His Val Val Tyr
145 150 155 160

Leu Leu Lys Lys Glu Gln Phe Gly Asn Gln Val Cys Gly Leu Ser Asp
165 170 175

Asp Glu Ile Glu Trp Gln Met Ala Pro Tyr Glu Asn Lys Ala Arg Leu
180 185 190

Arg Asp Phe Pro Gly Ser Tyr Lys His Pro Lys Tyr Leu Glu Leu Ile
195 200 205

Leu Leu Phe Asp Gln Ser Arg Tyr Arg Phe Val Asn Asn Asn Leu Ser
210 215 220

Gln Val Ile His Asp Ala Ile Leu Leu Thr Gly Ile Met Asp Thr Tyr
225 230 235 240

Phe Gln Asp Val Arg Met Arg Ile His Leu Lys Ala Leu Glu Val Trp
245 250 255

Thr Asp Phe Asn Lys Ile Arg Val Gly Tyr Pro Glu Leu Ala Glu Val
260 265 270

Leu Gly Arg Phe Val Ile Tyr Lys Lys Ser Val Leu Asn Ala Arg Leu
275 280 285

Ser Ser Asp Trp Ala His Leu Tyr Leu Gln Arg Lys Tyr Asn Asp Ala
290 295 300

Leu Ala Trp Ser Phe Gly Lys Val Cys Ser Leu Glu Tyr Ala Gly Ser
305 310 315 320

Val Ser Thr Leu Leu Asp Thr Asn Ile Leu Ala Pro Ala Thr Trp Ser
325 330 335

Ala His Glu Leu Gly His Ala Val Gly Met Ser His Asp Glu Gln Tyr
340 345 350

Cys Gln Cys Arg Gly Arg Pro Asn Cys Ile Met Gly Ser Gly Arg Thr
355 360 365

Gly Phe Ser Asn Cys Ser Tyr Ile Ser Phe Phe Lys His Ile Ser Ser
370 375 380

Gly Ala Thr Cys Leu Asn Asn Ile Pro Gly Leu Gly Tyr Val Leu Lys
385 390 395 400

Ser Thr Glu Glu Cys Gln Lys Asp Arg Cys Cys Gln Ser Asn Cys Lys
420 425 430

Leu Gln Pro Gly Ala Asn Cys Ser Ile Gly Leu Cys Cys His Asp Cys
435 440 445

Arg Phe Arg Pro Ser Gly Tyr Val Cys Arg Gln Glu Gly Asn Glu Cys
450 455 460

Asp Leu Ala Glu Tyr Cys Asp Gly Asn Ser Ser Ser Cys Pro Asn Asp
465 470 475 480

Val Tyr Lys Gln Asp Gly Thr Pro Cys Lys Tyr Glu Gly Arg Cys Phe
485 490 495

Arg Lys Gly Cys Arg Ser Arg Tyr Met Gln Cys Gln Ser Ile Phe Gly
500 505 510

Pro Asp Ala Met Glu Ala Pro Ser Glu Cys Tyr Asp Ala Val Asn Leu
515 520 525

Ile Gly Asp Gln Phe Gly Asn Cys Glu Ile Thr Gly Ile Arg Asn Phe
530 535 540

Lys Lys Cys Glu Ser Ala Asn Ser Ile Cys Gly Arg Leu Gln Cys Ile
545 550 555 560

Asn Val Glu Thr Ile Pro Asp Leu Pro Glu His Thr Thr Ile Ile Ser
565 570 575

Thr His Leu Gln Ala Glu Asn Leu Met Cys Trp Gly Thr Gly Tyr His
580 585 590

Leu Ser Met Lys Pro Met Gly Ile Pro Asp Leu Gly Met Ile Asn Asp
595 600 605

Gly Thr Ser Cys Gly Glu Gly Arg Val Cys Phe Lys Lys Asn Cys Val
610 615 620

Asn Ser Ser Val Leu Gln Phe Asp Cys Leu Pro Glu Lys Cys Asn Thr
625 630 635 640

Arg Gly Val Cys Asn Asn Arg Lys Asn Cys His Cys Met Tyr Gly Trp
645 650 655

Ala Pro Pro Phe Cys Glu Glu Val Gly Tyr Trp Gly Ser Ile Asp Ser
660 665 670

Gly Pro Pro Gly Leu Leu Arg Gly Ala Ile Pro Ser Ser Ile Trp Val
675 680 685

Val Ser Ile Ile Met Phe Arg Leu Ile Leu Leu Ile Leu Ser Val Val
690 695 700

Glu Lys Met Pro Leu Ser Lys Ala Lys Thr Glu Gln Glu Glu Ser Lys
725 730 735

Thr Lys Thr Val Gln Glu Glu Ser Lys Thr Lys Thr Gly Gln Glu Glu
740 745 750

Ser Glu Ala Lys Thr Gly Gln Glu Glu Ser Lys Ala Lys Thr Gly Gln
755 760 765

Glu Glu Ser Lys Ala Asn Ile Glu Ser Lys Arg Pro Lys Ala Lys Ser
770 775 780

Val Lys Lys Gln Lys Lys
785 790

<210> 16
<211> 781
<212> PRT
<213> Homo sapiens

<400> 16
Met Arg Ser Val Gln Ile Phe Leu Ser Gln Cys Arg Leu Leu Leu Leu
1 5 10 15

Leu Val Pro Thr Met Leu Leu Lys Ser Leu Gly Glu Asp Val Ile Phe
20 25 30

His Pro Glu Gly Glu Phe Asp Ser Tyr Glu Val Thr Ile Pro Glu Lys
35 40 45

Leu Ser Phe Arg Gly Glu Val Gln Gly Val Val Ser Pro Val Ser Tyr
50 55 60

Leu Leu Gln Leu Lys Gly Lys Lys His Val Leu His Leu Trp Pro Lys
65 70 75 80

Arg Leu Leu Leu Pro Arg His Leu Arg Val Phe Ser Phe Thr Glu His
85 90 95

Gly Glu Leu Leu Glu Asp His Pro Tyr Ile Pro Lys Asp Cys Asn Tyr
100 105 110

Met Gly Ser Val Lys Glu Ser Leu Asp Ser Lys Ala Thr Ile Ser Thr
115 120 125

Cys Met Gly Gly Leu Arg Gly Val Phe Asn Ile Asp Ala Lys His Tyr
130 135 140

Gln Ile Glu Pro Leu Lys Ala Ser Pro Ser Phe Glu His Val Val Tyr
145 150 155 160

Leu Leu Lys Lys Glu Gln Phe Gly Asn Gln Val Cys Gly Leu Ser Asp

Arg Asp Phe Pro Gly Ser Tyr Lys His Pro Lys Tyr Leu Glu Leu Ile
195 200 205

Leu Leu Phe Asp Gln Ser Arg Tyr Arg Phe Val Asn Asn Asn Leu Ser
210 215 220

Gln Val Ile His Asp Ala Ile Leu Leu Thr Gly Ile Met Asp Thr Tyr
225 230 235 240

Phe Gln Asp Val Arg Met Arg Ile His Leu Lys Ala Leu Glu Val Trp
245 250 255

Thr Asp Phe Asn Lys Ile Arg Val Gly Tyr Pro Glu Leu Ala Glu Val
260 265 270

Leu Gly Arg Phe Val Ile Tyr Lys Lys Ser Val Leu Asn Ala Arg Leu
275 280 285

Ser Ser Asp Trp Ala His Leu Tyr Leu Gln Arg Lys Tyr Asn Asp Ala
290 295 300

Leu Ala Trp Ser Phe Gly Lys Val Cys Ser Leu Glu Tyr Ala Gly Ser
305 310 315 320

Val Ser Thr Leu Leu Asp Thr Asn Ile Leu Ala Pro Ala Thr Trp Pro
325 330 335

Ala His Glu Leu Gly His Ala Val Gly Met Ser His Asp Glu Gln Tyr
340 345 350

Cys Gln Cys Arg Gly Arg Leu Asn Cys Ile Met Gly Ser Gly Arg Thr
355 360 365

Gly Phe Ser Asn Cys Ser Tyr Ile Ser Phe Phe Lys His Ile Ser Ser
370 375 380

Gly Ala Thr Cys Leu Asn Asn Ile Pro Gly Leu Gly Tyr Val Leu Lys
385 390 395 400

Arg Cys Gly Asn Lys Ile Val Glu Asp Asn Glu Glu Cys Asp Cys Gly
405 410 415

Ser Thr Glu Glu Cys Gln Lys Asp Arg Cys Cys Gln Ser Asn Cys Lys
420 425 430

Leu Gln Pro Gly Ala Asn Cys Ser Ile Gly Leu Cys Cys His Asp Cys
435 440 445

Arg Phe Arg Pro Ser Gly Tyr Val Cys Arg Gln Glu Gly Asn Glu Cys
450 455 460

Asp Leu Ala Glu Tyr Cys Asp Gly Asn Ser Ser Ser Cys Pro Asn Asp
465 470 475 480

Arg Lys Gly Cys Arg Ser Arg Tyr Met Gln Cys Gln Ser Ile Phe Gly
500 505 510

Pro Asp Ala Met Glu Ala Pro Ser Glu Cys Tyr Asp Ala Val Asn Leu
515 520 525

Ile Gly Asp Gln Phe Gly Asn Cys Glu Ile Thr Gly Ile Arg Asn Phe
530 535 540

Lys Lys Cys Glu Ser Ala Asn Ser Ile Cys Gly Arg Leu Gln Cys Ile
545 550 555 560

Asn Val Glu Thr Ile Pro Asp Leu Pro Glu His Thr Thr Ile Ile Ser
565 570 575

Thr His Leu Gln Ala Glu Asn Leu Met Cys Trp Gly Thr Gly Tyr His
580 585 590

Leu Ser Met Lys Pro Met Gly Ile Pro Asp Leu Gly Met Ile Asn Asp
595 600 605

Gly Thr Ser Cys Gly Glu Gly Arg Val Cys Phe Lys Lys Asn Cys Val
610 615 620

Asn Ser Ser Val Leu Gln Phe Asp Cys Leu Pro Glu Lys Cys Asn Thr
625 630 635 640

Arg Gly Val Cys Asn Asn Arg Lys Asn Cys His Cys Met Tyr Gly Trp
645 650 655

Ala Pro Pro Phe Cys Glu Glu Val Gly Tyr Gly Gly Ser Ile Asp Ser
660 665 670

Gly Pro Pro Gly Leu Leu Arg Gly Ala Ile Pro Ser Ser Ile Trp Val
675 680 685

Val Ser Ile Ile Met Phe Arg Leu Ile Leu Leu Ile Leu Ser Val Val
690 695 700

Phe Val Phe Phe Arg Gln Val Ile Gly Asn His Leu Lys Pro Lys Gln
705 710 715 720

Glu Lys Met Pro Leu Ser Lys Ala Lys Thr Glu Gln Glu Gln Ser Lys
725 730 735

Thr Lys Thr Val Gln Glu Glu Ser Lys Thr Lys Thr Gly Gln Glu Glu
740 745 750

Ser Glu Ala Lys Thr Gly Gln Glu Glu Ser Lys Ala Asn Ile Glu Ser
755 760 765

Lys Arg Pro Lys Ala Lys Ser Val Lys Lys Gln Lys Lys
770 775 780

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 17
cacctaaggt gttcaatttt ttg 23

<210> 18
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 18
cadatactgc aagtgagact tgc 23

<210> 19
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 19
tgcacaacta cgtgtggtgt accc 24

<210> 20
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 20
gagccactgc aattgaaaaa gtgccc 26

<210> 21
<211> 21
<212> DNA
<213> Artificial Sequence

<400> 21
aatgatgctc ttgcatggtc g 21

<210> 22
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 22
ctttcacgga gcccatgtag ttgcag 26

<210> 23
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 23
tgaaggagaa aacgcgcaga tgtcgg 26

<210> 24
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 24
tcgataatgc atgaaggcaa cccacc 26

<210> 25
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 25
caagtctcac ttgcagtatt tgcgcc 26

<220>
<223> Description of Artificial Sequence: primer

<400> 26
gccactgcat gtatgggtg 19

<210> 27
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 27
gacactcttt gctttgggtc g 21

<210> 28
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: peptide fragment

<400> 28
Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

<210> 29
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: peptide fragment

<400> 29
Lys Asp Val Ala Ser Leu Arg Gln Gln Val Gln Ala Leu Gln Thr Gln
1 5 10 15

Val Gln His Leu Gln Ala Ala Phe Ser Gln Tyr
20 25

<210> 30
<211> 33

<220>
<223> Description of Artificial Sequence: peptide fragment

<400> 30
Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile
1 5 10 15

Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu
20 25 30

Arg

<210> 31
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: peptide fragment

<220>
<223> "Xaa" at various positions throughout the sequence may be any amino acid

<400> 31
His Glu Xaa Xaa His Xaa Xaa Gly Xaa Xaa His Asp
1 5 10

<210> 32
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: peptide fragment

<400> 32
Ser Gln Ser Gln Pro Pro Leu Met Pro
1 5

<210> 33
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: peptide

<400> 33
Gln Glu Glu Ser Lys Xaa Lys Thr Gly
1 5